

THE  
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EDITOR  
HENRY CHANDLER COWLES

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WITH NINE PLATES AND TWO HUNDRED FORTY-TWO FIGURES



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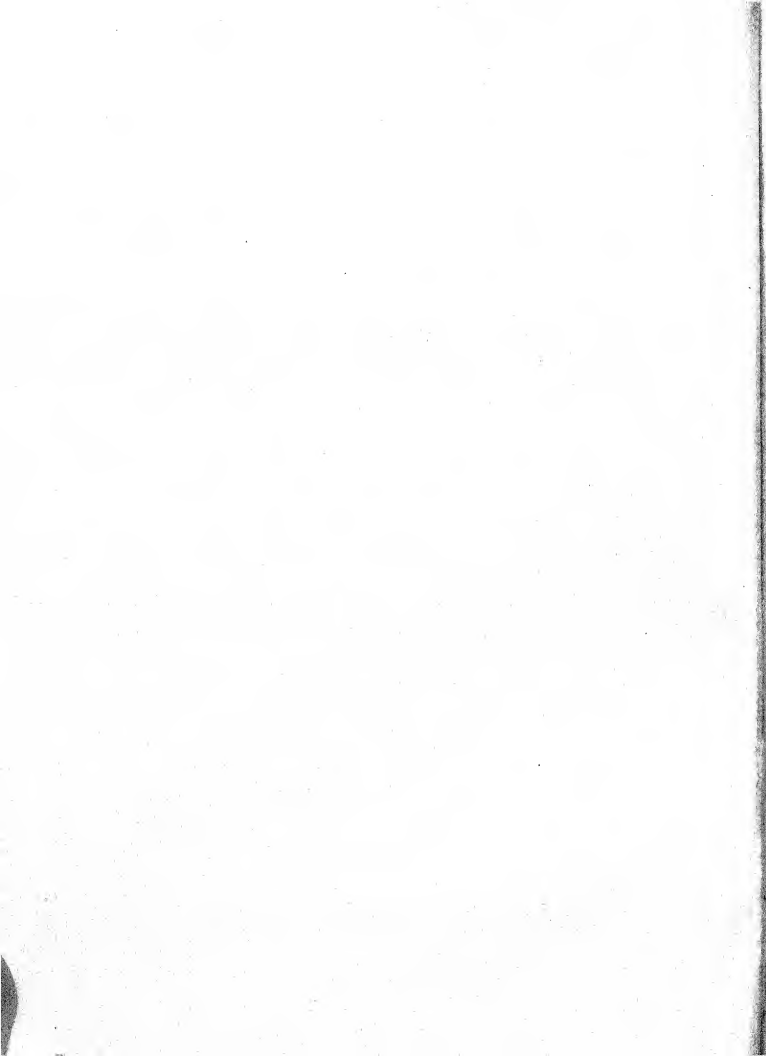
## ERRATA

## VOL. LXXXVIII

- P. 51, line 1, for "different spermatozoids" read "spermatozoids from different gametophytes"
- P. 51, line 5, for "by eggs" read "by spores derived from eggs"
- P. 355, line 8, for "continues" read "continuous"

## VOL. LXXXIX

- P. 118, line 7, for "increase in diameter" read "increase in radius"
- P. 123, line 6, for "3.5 mm. in diameter" read "3.5 mm. in radius"



# THE BOTANICAL GAZETTE

March 1930

## COMPARATIVE ANATOMY AND MORPHOLOGY OF EMBRYOS AND SEEDLINGS OF MAIZE, OATS, AND WHEAT

GEORGE S. AVERY, JR.

(WITH SIXTY-EIGHT FIGURES)

### Introduction

The structures of the embryos and the developmental anatomy of the seedlings of *Zea mays* L., *Avena sativa* L., and *Triticum vulgare* Vill. were selected for this study, since they are representative of the three morphological types of Gramineae distinguished by VAN TIEGHEM (27). Attention was paid to possible homologies of structure in embryos, in light of their later development; particularly to that part of the axis which in the seedling lies between the level at which the scutellum appears to be attached and the level of divergence of the coleoptile.

### EMBRYO

Earlier literature includes many extensive and diverse studies on grass embryos. From observations on the embryos of rye and a few other Gramineae, SCHLEIDEN (21) concluded that the scutellum is the lateral limb or spreading part of the cotyledon, the coleoptile being the ligule. He was apparently the first to note that the coleoptile is a lateral structure. Although the epiblast is shown in his figures, it was seemingly regarded as of little morphological importance.

In the same year DE JUSSIEU (14) published his observations on several monocotyledons other than those studied by SCHLEIDEN, in-

cluding *Canna*. He interpreted the coleoptile as the true cotyledon, his argument being based principally on the fact that one embryonic leaf sheathes another, the coleoptile being the sheathing leaf that covers all the other embryonic leaves. He considered the scutellum to be a lateral outgrowth of the young axis which has the appearance of a cotyledon. He further considered it as playing the physiological rôle of a cotyledon. In fact, so often does it play this rôle that he considered the coleoptile imperfect and reduced to the state of a sheath.

LESTIBOUDOIS (15), studying the anatomical insertion of the first leaves of Gramineae, and those of maize in particular, considered the coleoptile to be the cotyledon. Studies on wheat, *Canna*, and other monocotyledons led him to the same conclusion. Substantially, then, he agreed with DE JUSSIEU. SCHACHT (20), studying species of *Agropyron*, agreed in part with SCHLEIDEN in considering the scutellum to be the cotyledon. However, he considered the coleoptile to be the second leaf of the plant.

Although GRIS (12) suggested no homologies in connection with the scutellum, he did describe it:

By its great development, by its relations with the perisperm, by its connections with the embryo, by its anatomical relation during germination, it is the principal organ of absorption for the embryo, a sort of neutral medium between a structure being absorbed and an organism which becomes developed.

The first extensive observations to be made on Gramineae were those of VAN TIEGHEM (26). He examined several genera, and differed from his predecessors in that he interpreted the grass embryo in terms of its development. The cotyledon was to him made up of the scutellum and coleoptile, the former being absorptive and the latter of the nature of a bistipular sheath. He considered the epiblast of no morphological importance. Later VAN TIEGHEM (27) renounced many of his former views, and considered the scutellum as the first cotyledon, the epiblast representing the rudimentary second cotyledon. He further considered the coleoptile to be the two-bundled and sheathing third leaf, superposed to the first leaf or scutellum. The first green leaf, many-bundled, with open sheath surmounted by a blade, and superposed to the second leaf (epiblast), represented the fourth leaf of the plant.

HEGELMAIER (13), having worked with wheat and other grasses as well as other monocotyledons, agreed with VAN TIEGHEM's earlier interpretation in considering the cotyledon to be composed of the scutellum and coleoptile. BRUNS (4) regarded the scutellum as the functional cotyledon; the epiblast as a rudimentary one.

SCHLICKUM (22) regarded the scutellum and coleoptile as together representing the cotyledon. CELAKOVSKÝ (7) agreed in part with the early work of VAN TIEGHEM. He considered the scutellum as homologous with the blade of a grass leaf, the sheath which incases the bud (the coleoptile) corresponding to the closed ligule of a grass leaf; later, after growth begins, the latter appears like a free axillary double stipule. The epiblast was considered to be an appendage of the scutellum, representing the auricles at the base of each foliage leaf in certain grasses.

CANNON (6), studying the embryogeny of *Avena fatua*, treated the scutellum as the cotyledon and analogous to the haustoria of certain parasites of flowering plants. The coleoptile was interpreted as a ligule-like outgrowth arising from the base of the cotyledon, and homologous with the ligule of the grass blade. He further regarded the epiblast as a second cotyledon, although not homologous with a true cotyledon. He showed the epiblast arising as an outgrowth of the upper part of the coleorhiza.

COULTER (9) contended that, in the evolution of monocotyledons from dicotyledons, there was a suppression of one cotyledon. He cited maize and members of other monocotyledonous families as showing that the epiblast is a second cotyledon which attains only a rudimentary stage of development. The scutellum represented the one remaining functional cotyledon.

SARGANT and ARBER (18), after an extensive study of the embryos of several genera of grasses, concluded that the cotyledon consists of a sucking apex (the scutellum), and a sheath (the coleoptile), the latter being perhaps equivalent to a pair of stipules. This "double" interpretation of the coleoptile apparently was abandoned later (ARBER 1). They (18) considered the epiblast as an outgrowth of the cotyledon, of the axis, or of both, and as being of little morphological importance. Although changing this interpretation as to the importance of the epiblast (1), ARBER (2) still later returned to

the earlier interpretation of SARGANT and ARBER (18). WORSDELL (30), in observations on maize, barley, and other grasses, concluded that the scutellum is the blade of the cotyledon, corresponding to that of the foliage leaf of the grass. He considered the epiblast part of the cotyledon, representing the auricles which occur at the base of the blade of the foliage leaf of certain grasses, in agreement with CELAKOVSKÝ (7). BUGNON (5) regarded the epiblast as a cotyledonary leaf which is habitually atrophied, the first leaf of the plant. He further regarded the scutellum as representing the functional cotyledon, alternate distichous with the first vegetative leaf. The coleoptile was interpreted as the cotyledonary sheath.

PERCIVAL (16), reporting on wheat, considered the scutellum to be the cotyledon, constituting, with the epiblast and coleoptile, the first three leaves of the plant. SOUÈGES (23), studying the embryogeny of *Poa annua*, considered the scutellum to be the cotyledon, the coleoptile (in part) being formed from it. He further showed that the epiblast arises as an outgrowth of the coleorhiza. TOOLE (25), although not working mainly on the structure of the embryo, agreed substantially with SARGANT and ARBER. His observations were on maize.

#### SEEDLING

The opinions of earlier workers already cited concern only the structure of the embryo. Those now referred to deal with the seedling, in particular with that part of the axis which in maize lies between the scutellum underground, and the node at the surface of the ground where the coleoptile diverges from the axis. The corresponding structure in oats is equally prominent, while that in wheat is somewhat less conspicuous and will be referred to later.

Apparently the first reference to this structure was by CLOS (8). Although working chiefly on monocotyledons other than Gramineae, he described the seedling of maize as follows:

That part of the axis between the attachment of the scutellum and the point of insertion of the coleoptile has a certain development. It presents a pith, a fibrovascular circle, and a cortical zone. It is a structure peculiar to monocotyledons, and maize in particular. The true stem seems to begin above the point of insertion of the cotyledonary sheath, where numerous bundles appear in the midst of the parenchyma.

He referred to this part of the axis only as the "neck."

VAN TIEGHEM (26) was the first to give particular attention to the structure. He regarded it as an elongated first node, however, belonging neither to the epicotyl nor to the hypocotyl. His argument was based on the fact that, in maize and similar types of grasses, there is an interval on the axis between the attachment of the coleoptile and that of the scutellum; the former being superposed directly above the latter and interpreted as the sheath of the cotyledon (scutellum). VAN TIEGHEM (27) renounced many of his former views on grass morphology, and interpreted the scutellum, epiblast, coleoptile, and first foliage leaf as the first four leaves of the plant.

DEBARY (10), reporting on maize, states that "the elongated internode between the insertion of the scutellum and the first sheathing leaf in the seedling of *Zea mays* shows an abnormal arrangement." Apparently he was the first to consider the interval in question as the first internode of the axis. BRUNS (4), studying several genera, including maize, also interpreted the structure as an internode. CELAKOVSKÝ (7), however, in complete agreement with VAN TIEGHEM's earlier interpretation, held that the structure in question is of the nature of an elongated first node, belonging neither to the epicotyl nor to the hypocotyl. He applied the term "mesocotyl," which has since been in rather common use.

SARGANT and ARBER (18) considered the "mesocotyl" as unique. They regarded it as having resulted from a fusion of the cotyledonary stalk with the hypocotyl, and thus neither a node nor an internode. COULTER (9) was in complete agreement with BRUNS. WORSDELL (30) agreed with VAN TIEGHEM (26) as to the nature of the structure. BUGNON (5) apparently agreed with the interpretation of CELAKOVSKÝ. WEATHERWAX (29) called the structure in question an internode, but did not discuss it further. PERCIVAL (16) referred to it briefly as a "rhizome."

### Materials and methods

The greater part of the maize (*Zea mays*) used in this experiment was of the Golden Glow variety, grown at the experimental farms of the University of Wisconsin. Other varieties studied were Golden Bantam and Ninety-Day Yellow Dent. In addition, Dwarf, Liguleless, and Chinese Suckering strains were observed. The material was

grown in 1925. The oats principally used were *Avena sativa*, although *A. fatua*, *A. strigosa*, *A. sterilis*, and *A. brevis* were examined. These were obtained from the United States Department of Agriculture, Bureau of Plant Industry. The wheat chiefly used was Turkey Red (*Triticum vulgare*), a hard winter wheat, grown in 1924-25. Less extensive observations were made on Durum and Einkorn, grown in 1921. The wheats came from the experimental farms of the University of Wisconsin.

Studies were made on the material as it grew in the greenhouse and in the field. The material taken for microscopic study came from both sources, and was fixed in formal-acetic-alcohol. Many of the seedlings were dehydrated and cleared in xylol for purposes of gross observation. The material for sectioning was imbedded by the paraffin method and sectioned at a thickness of 12  $\mu$ .

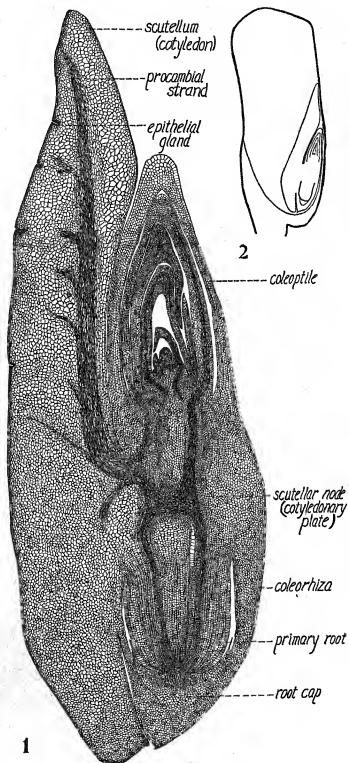
In the studies on embryos, serial sections in every case were cut in transverse, longitudinal-side, and longitudinal-face sections. Conclusions were based largely on evidence gained from germinating embryos and seedlings. In addition to the embryos and seedlings of maize, oats, and wheat, those of barley, rye, and rice were observed. Although these differ structurally, they were found to agree with the interpretation reached from the study of maize, oats, and wheat.

### Maize

#### STRUCTURE OF EMBRYO

The embryo lies imbedded in the endosperm (fig. 2), on one side and toward the base of the caryopsis. The lower end of the embryo is always nearest the narrowed end of the caryopsis. In longitudinal-side section (fig. 1) the upper part of the axis may be seen to consist of nodes bearing the primordia of leaves. The internodes are unelongated. There is a short necklike interval in the axis between the levels of divergence of the embryonic leaves and that of the scutellum. The apex of the axis consists of a small growing point. The embryonic leaves and growing point are covered by the coleoptile, making an approximately cone-shaped structure. In longitudinal-face section (fig. 10) it is possible to observe the primordia of two or more adventitious roots arising in the region just above the scutellar node. They are directed laterally upward.





FIGS. 1, 2.—*Zea mays*: fig. 1, median longitudinal-side section of embryo; fig. 2, diagram of median longitudinal-side section of caryopsis, showing location of embryo.

The scutellum is a prominent structure attached to one side of the axis. It is largely parenchymatous, and has a prominent layer of epithelial cells in contact with the endosperm tissue during germination (fig. 1). The epithelial layer often has infoldings in its surface, which have been described by SARGANT and ROBERTSON (19) as epithelial glands.

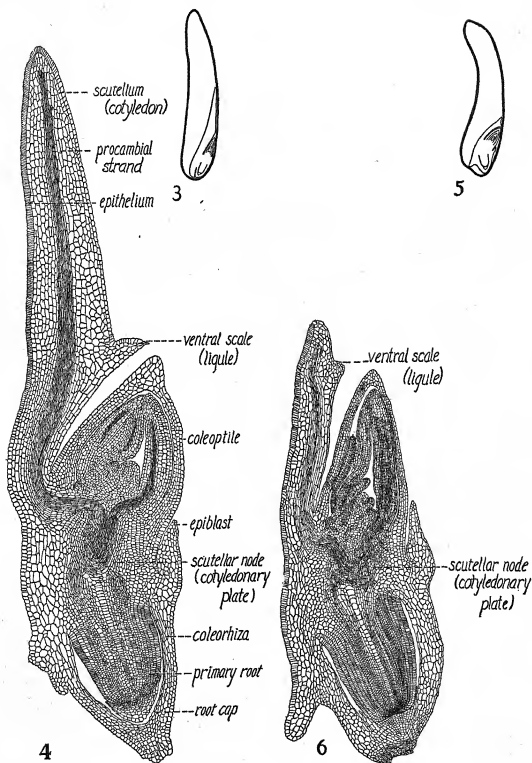
The embryonic vascular system consists partly of a large procambial strand laid down in the scutellum. This main procambial bundle branches throughout its length (figs. 1, 7). In the lower part of the scutellum are several procambial strands which radiate out and downward from the level where they diverge from the axis. In longitudinal-face section they appear much like the ribs of an inverted fan (fig. 7).

The procambial bundles from the upper and lower ends of the scutellum are joined at the level of divergence of the scutellum from the axis, and within the embryonic axis are connected directly with the stele (figs. 1, 7, 36). Continuing from the place of attachment of the scutellum bundle to the stele, it is easily possible to trace procambial elements to the first leaf above the coleoptile (fig. 1). The vascular system of the coleoptile consists usually of two vascular bundles, one on either side. They arise directly from the stele of the embryonic axis, at the upper end of the necklike interval in the axis already referred to (figs. 10, 36). They will be more fully described later.

#### GERMINATION

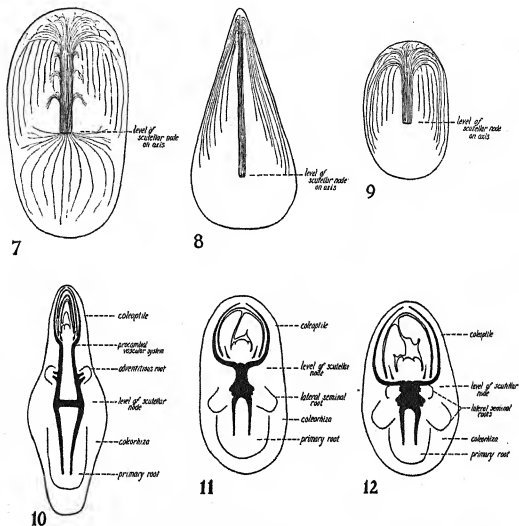
Under conditions favorable for germination, the coleorhiza bursts through the pericarp and is soon penetrated by the primary root, which grows rapidly downward (figs. 13, 14). Soon after the emergence of the coleorhiza the coleoptile emerges, the growing point of the stem together with embryonic leaves being inclosed within it. The coleoptile is pushed upward in consequence of an elongation of that part of the axis just below the level of its divergence (figs. 15, 16, 18, 19). The coleoptile remains closed until its base is level with or slightly below the surface of the soil; then its edges spread apart near its tip, on the side opposite the scutellum (fig. 16). Subsequent foliage leaves soon appear (fig. 17).

The adventitious roots, described as directed laterally upward in



FIGS. 3-6.—*Avena sativa*: fig. 3, diagram of median longitudinal-side section of caryopsis, showing location of embryo; fig. 4, median longitudinal-side section of embryo; fig. 5, *Triticum vulgare*, median longitudinal-side section of caryopsis, showing location of embryo; fig. 6, *T. vulgare*, median longitudinal-side section of embryo.

the embryo, appear early in germination (fig. 15). Although at first growing upward, they soon respond positively to the stimulus of gravity. About ten days after germination, a whorl of four or more

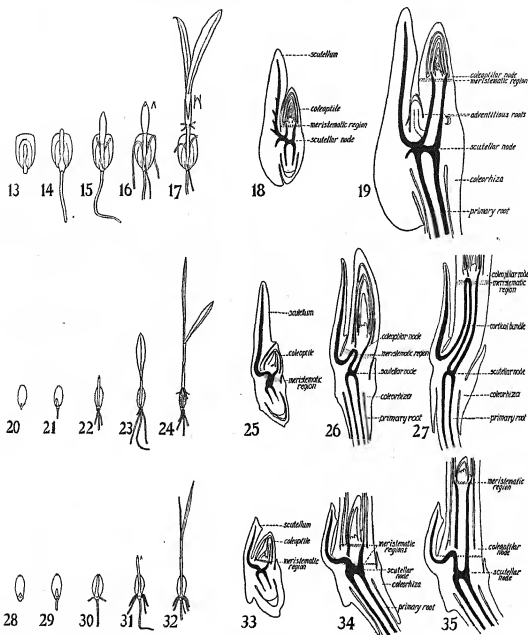


FIGS. 7-12.—Figs. 7-9, face view diagrams of scutella of *Zea mays*, *Avena sativa*, and *Triticum vulgare* respectively, showing procambial vascular systems; figs. 10-12, face view diagrams of embryonic axes of *Z. mays*, *A. sativa*, and *T. vulgare* respectively, showing procambial vascular systems in black.

roots arises from the axis just above the level of divergence of the coleoptile (fig. 17).

#### ANATOMY OF SEEDLING

**SEMINAL ROOTS.**—The primary root has a pith composed of large parenchymatous cells, isodiametric in transverse section, constituting the larger portion of the stele (fig. 57). The pith cells about the



FIGS. 13-35.—Figs. 13-19, *Zea mays*: figs. 13-17, stages of development in germination; fig. 18, median longitudinal-side section of dormant embryo (region in which first axial elongation will begin shown in dashes); fig. 19, germinating embryo, 24 hours old, showing elongation of axis between level of attachment of scutellum and that of coleoptile; figs. 20-27, *Avena sativa*: figs. 20-24, stages of development in germination; fig. 25, median longitudinal-side section of dormant embryo (region in which first axial elongation will begin shown in dashes); fig. 26, germinating embryo, 36 hours old, showing beginning of elongation; fig. 27, same, 5 days old; figs. 28-35, *Triticum vulgare*: figs. 28-32, stages of development in germination; fig. 33, median longitudinal-side section of dormant embryo (region in which principal elongation of axis will take place shown in dashes); fig. 34, same, 7 days old (slight elongation of axis just above scutellar node is starting to take place, also shown in dashes); fig. 35, same, month-old plant showing elongated second internode sheathed by coleoptile; first internode in this case shows unusual elongation.

metaxylem vessels become thick-walled within ten days after germination. The number of protoxylem points varies from eighteen to more than twice that number. There is usually one large metaxylem vessel for each two or three protoxylem groups. Alternating with the protoxylem groups are groups of phloem, each usually composed of several cells. The pericycle is characteristically a single layer of cells. Most of the cells of the endodermis have their entire inner tangential walls conspicuously thickened. The epidermis is lost early, and the walls of the outer few layers of cortical cells often become thickened. This thickening does not take place to any marked degree, however, until after the root hairs have become functionless; it is simultaneous with the thickening of the walls of certain cells of the pith, already described. Anatomically the roots arising from the axis just above the level of attachment of the scutellum are similar to the primary root. They differ in being smaller and in having fewer protoxylem points.

The structure of the first adventitious roots to appear at the surface of the ground is essentially the same as that of earlier seminal roots. They become much larger, however, and frequently have as many as forty-eight or more protoxylem points.

**SEEDLING AXIS.**—The first step in the "transition" from the exarch condition of the xylem of the root to the endarch condition of the xylem of the stem takes place in the vascular plate at the scutellar node (figs. 19, 42). In addition, the interval in the axis between the level of divergence of the scutellum and that of the coleoptile is a transition region. Exarch xylem groups may be observed as far up as the divergence of the coleoptile (fig. 58).

The axis above the scutellum is partly sheathed at its lower end by the scutellum (fig. 45). In transverse section (fig. 58) it resembles the primary root (fig. 57) in having small exarch xylem groups. There are, however, fewer of these groups. There is a large pith of thin-walled isodiametric parenchymatous cells. In addition, between the exarch xylem groups are endarch collateral bundles whose phloem alternates with the exarch xylem groups. The collateral bundles are not as sharply defined as are those in the upper internodes (fig. 60). The endarch and exarch bundles are arranged in a circle (transverse section in fig. 58) just inside the periphery of the

stele, similar to the circle of vascular tissue in the root. Ordinarily exarch groups and endarch bundles occur alternately, or there may be two or more endarch bundles separating successive exarch groups. The pericycle is easily identified as the prominent layer of cells immediately within the endodermis. Adventitious roots may arise from the pericycle at any level between the scutellum and the coleoptile. The radial and inner tangential walls of the endodermal cells are heavily thickened. The cortex is several cells in thickness; surrounding it is a layer of epidermal cells whose outer walls are lightly cutinized. The walls of one or more layers of cortical cells beneath the epidermis are often slightly thickened.

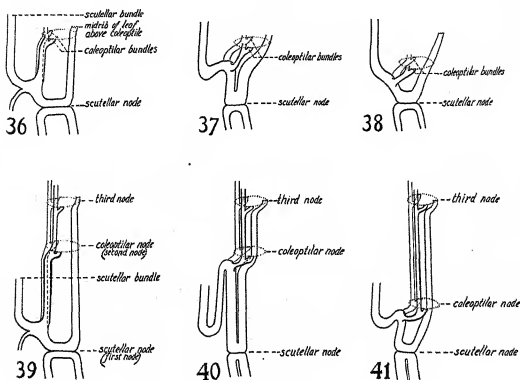
The interval on the axis between the scutellum and the coleoptile elongates by means of division and enlargement of cells just below the level of divergence of the coleoptile (figs. 18, 19). This differs from the higher internodes, which elongate as a result of activity of intercalary meristems near their base, rather than their top.

The vascular supply of the coleoptile diverges from two bundles that occur on the side of the stele toward the scutellum (figs. 36, 39). At the level of the coleoptilar node, approximately half of each of these bundles diverges laterally outward into the coleoptile. The remaining half of each bundle continues upward in the axis, giving rise to one or more traces to the leaf above the coleoptile (fig. 39). Only minor variations have been observed to take place in the origin of the vascular supply of the coleoptile and of the leaf next above it. It is obvious that the bundles of the coleoptile are ordinary leaf traces from the stele. The internode between the coleoptile and the foliage leaf next above it is sheathed by the coleoptile (fig. 48), and differs anatomically from the higher internodes (fig. 59). It possesses a rather thick cortex, very indefinite and poorly defined bundles, and an almost continuous sheath of meristematic cells among which may be found some differentiated xylem and phloem. While no particular study has been made of this meristematic sheath, it would appear to be of pericyclic origin. The internodes above the second and third foliage leaves resemble more closely the higher internodes (fig. 60).

**COLEOPTILE.**—The coleoptile has already been described as sheathing the growing point and embryonic leaves during the early stages of germination, and as being elevated by the growth of the

elongating axis. It has also been described as having commonly a vascular system composed of two bundles, one on either side (fig. 51). The origin of the coleoptile traces has been described.

There are exceptions to the usual number of vascular bundles in the coleoptile. DEBARY (10) notes that the two bundles usually present may each divide, making four bundles in all. This division, however, occurs before they diverge from the axis. The writer (3) has



FIGS. 36-41.<sup>1</sup>—Fig. 36, *Zea mays*: part of procambial vascular system of embryo, showing relation of scutellar bundle to vascular system of embryonic axis; fig. 37, *Avena sativa*, same; fig. 38, *Triticum vulgare*, same; fig. 39, *Zea mays*, showing only those bundles of seedling vascular system derived at least in part from scutellum bundle; fig. 40, *Avena sativa*, same; fig. 41, *Triticum vulgare*, same. More traces than are shown actually diverge from this source into leaf above coleoptile.

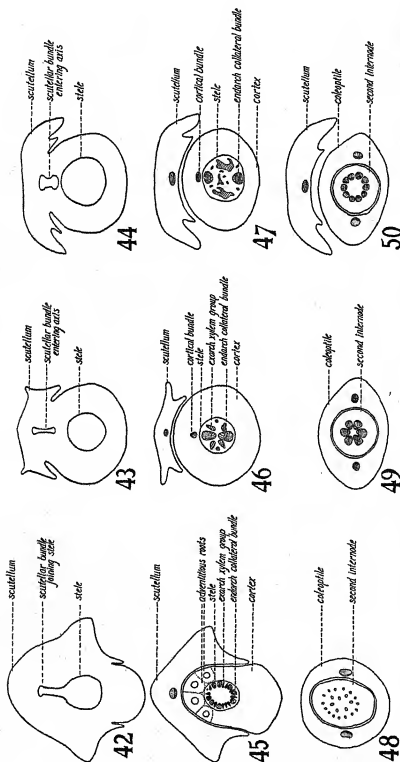
reported the presence of as many as five bundles. The extra bundles have ordinarily an origin similar to that of the usual two, although in the case of five, the fifth bundle usually arises *de novo* in the cortex below the coleoptilar node.

In transverse section the coleoptile displays a very simple struc-

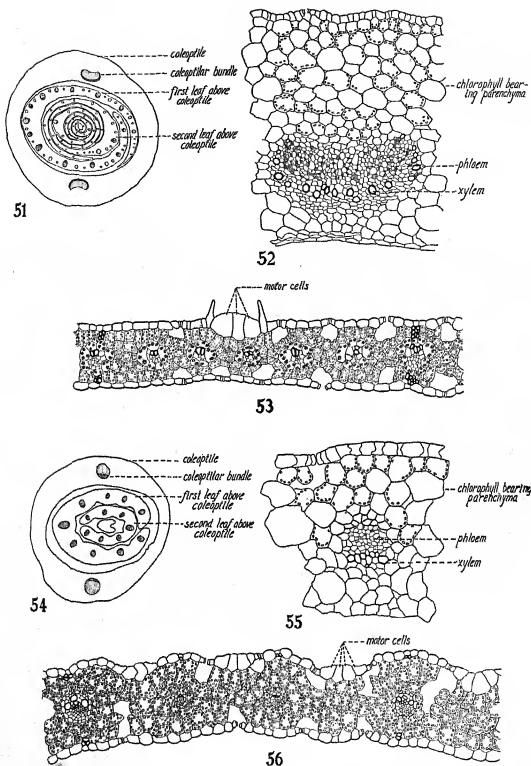
<sup>1</sup> The following diagrams are designed to show destination of bundles rather than minute details of their course.



ture, being largely parenchymatous (fig. 52). The vascular bundles are large. Each bundle has many xylem groups and a considerable



FIGS. 42-50.—Figs. 42-44, diagrams of transverse sections through axes of *Zea mays*, *Avena sativa*, and *Triticum vulgare* respectively, at level of scutellar (cotyledonary) node; figs. 45-47, same, through first internode, at level immediately above first node; figs. 48-50, same, of second internode, at level just above attachment of cotyledon.

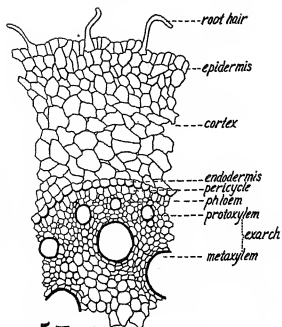


FIGS. 51-56.—Figs. 51-53, *Zea mays*: fig. 51, transverse section of coleoptile showing sheathing of later leaves; fig. 52, transverse section of small portion of coleoptile, including vascular bundle and surrounding tissue; fig. 53, transverse section of portion of later foliage leaf; figs. 54-56, *Triticum vulgare*: fig. 54, diagram of transverse section of coleoptile showing sheathing of later leaves; fig. 55, transverse section of small portion of coleoptile, including vascular bundle and surrounding tissue; fig. 56, transverse section of small portion of later foliage leaf.

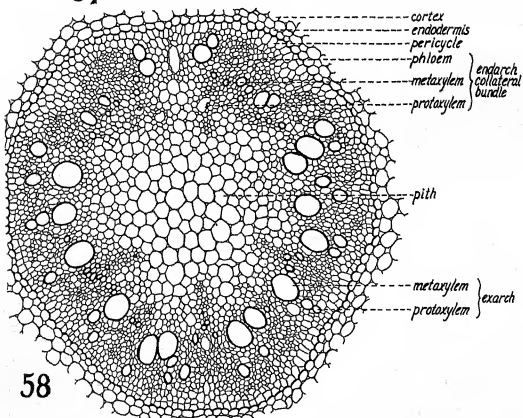
amount of phloem tissue. The parenchyma making up the body of the coleoptile is closely arranged, having only small intercellular spaces. Stomata are present only in the outer epidermis. They are usually confined to one or a few rows on either side of the two vascular bundles. The guard cells are small, as are the air spaces beneath them. Chloroplasts are most numerous in the region of the bundles.

A bud was present in the axil of the coleoptile in less than 1 per cent of the maize seedlings examined (AVERY 3). Its presence appears to depend largely upon moisture and temperature conditions at the time of germination.

LATER FOLIAGE LEAVES.—Structurally the later foliage leaves of maize are typically similar to an ordinary grass leaf, being composed of sheath and blade. The collar-like ligule is present in most strains of maize. There are, however, liguleless strains. The blade is typically parallel-veined, but smaller cross-connecting bundles are found between the larger veins. The leaf has large and small bundles (fig. 53), the large bundles occurring between groups of from two to eight small bundles, and differing from the latter both in size and in development of mechanical tissue above and below each bundle. The mechanical tissue associated with the midrib and the larger bundles is the chief support of the leaf. Transverse sections of the sheath and blade show only minor differences. The mesophyll consists of closely arranged cells, the spongy condition being uncommon (fig. 53). There is no well developed palisade layer. The vascular bundles are surrounded by a chlorophyll-bearing bundle sheath. The chloroplasts of the bundle sheath cells differ from those of other cells of the leaf (fig. 53). The epidermis, both upper and lower, is a prominent layer of cells, the lower epidermis being more heavily cutinized than the upper. In both epidermal layers the cells are nearly square in transverse section of the leaf, but in longitudinal section are much elongated. The upper epidermis is also characterized by the presence of conspicuous hygroscopic motor, or bulliform cells, which under dry conditions apparently constitute the mechanism that functions in the rolling of the leaf. These motor cells occur in rows, and have little or no cuticle. In addition, in most varieties of maize, there are hairs on either side of these longitudinal rows of motor cells. Stomata are present in both upper and lower epidermis, and

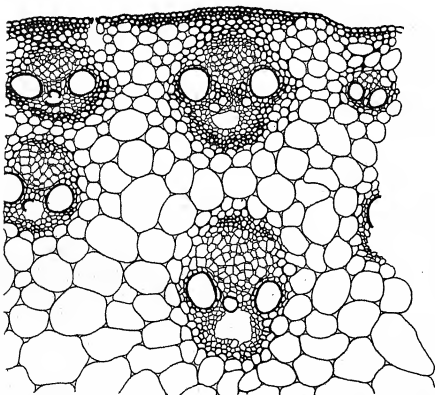
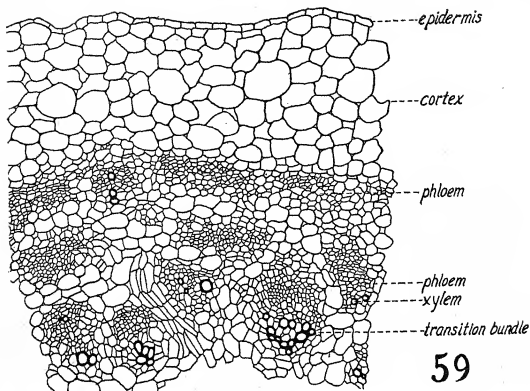


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FIGS. 57, 58.—*Zea mays*: fig. 57, transverse section of small portion of primary root of seedling; fig. 58, transverse section of stele in first internode of seedling.



FIGS. 59, 60.—*Zea mays*: fig. 59, transverse section of portion of second internode of 3-weeks-old plant; fig. 60, transverse section of portion of sixth internode of mature plant.

are arranged in rows parallel to the long axis. Epidermal cells alternate in rows with them, and under each stoma is a large intercellular space.

### Oats

#### STRUCTURE OF EMBRYO

The embryo lies imbedded in the endosperm slightly to one side of the base of the caryopsis (fig. 3). It is opposite the groove (crease). In general appearance it is similar to the embryo of maize, but much smaller (fig. 4). The scutellum much exceeds the embryonic axis in length, is slender, and has a sharply convex face toward the endosperm. As compared with the maize embryo, it differs in at least six respects: (1) There is an epiblast (fig. 4). (2) The primordia of the seminal roots other than the primary root are usually two in number. In face section they appear in, or slightly below the region of the scutellar node (fig. 11), being directed laterally downward. A third seminal root is often present opposite the scutellum, at about the same level as the lateral roots. (3) The large procambial bundle in the scutellum shows small branch strands diverging from the upper part of the main trunk, so that the scutellar vascular system has a pattern much like the ribs of a fan, with the tips bent over toward the handle (fig. 8). There is no vascular development in the lower part of the scutellum. (4) In the dormant embryo there is no elongated interval in the axis between the scutellum and the coleoptile. (5) The epithelium of the scutellum has not been observed to possess infoldings in its surface (fig. 4). (6) On the ventral face of the scutellum is the "ventral scale," a small protrusion of tissue a considerable distance down from the apex and slightly overhanging the coleoptile (fig. 4).

#### GERMINATION

Under usual conditions, structurally the external features of the germinating oat correspond with those of maize (figs. 20-24), except that there are but two adventitious roots in the region of the coleoptilar node. These are initiated just below the level of divergence of the coleoptile rather than above it, as in maize. A third may appear later, at a level just above the divergence of the coleoptile. Part of the anatomical changes during germination may be observed in figs.

## ANATOMY OF SEEDLING

**SEMINAL ROOTS.**—The primary root of the oat (fig. 61) is essentially like that of wheat (fig. 65). Structurally each primary root is characterized by a large central metaxylem vessel, which shows a lignified wall when a few days old. One or more smaller vessels may occur in the central region around the larger one, and these in turn are surrounded by parenchyma. The number of protoxylem points of the polyarch xylem varies, seven and eight being the most common numbers. Alternating with these groups are phloem areas of one to several cells each. The phloem is not well differentiated in the young seminal roots. The pericycle consists of a single layer of cells. The endodermis is characterized by thickened inner tangential walls. In the region of absorption, however, the walls of the endodermal cells opposite the xylem points are unthickened. The width of the cortex is about the same as the diameter of the stele. The epidermal cells are lightly cutinized on their outer tangential walls. As in maize, the epidermal layer is soon lost, and the layer of cortical cells next to it becomes conspicuously thickened. Anatomically the other seminal roots and the adventitious roots arising above the coleoptile are similar to the primary root.

**SEEDLING AXIS.**—The first step in the "transition" from the exarch to the endarch xylem condition takes place largely in the vascular plate at the scutellar node. The change is abrupt. Vascular differentiation at this level takes place as follows: When differentiation begins in the axis (during embryogeny), it takes place both upward and downward from the vascular plate. The upward differentiation results in a stele that is stemlike in part (figs. 46, 62), while downward differentiation results in typical root structure (fig. 61). Many of the xylem and phloem strands are continuous from root to stem. Above this abrupt transition region is a transition interval which extends from the level of divergence of the scutellum to that of the coleoptile. The elongation of this interval with a diagrammatic view of internal development may be observed in figs. 25-27. The location of the meristematic region in this interval is similar to that in maize. However, when elongation takes place it is seen to be slightly different, being above the vascular plate at the scutellar node and surrounding the scutellar bundle (fig. 25). Therefore the

scutellar bundle in the seedling axis extends upward parallel to, but does not become part of, the stele until it reaches the level of divergence of the coleoptile (fig. 27).

At the level of divergence of the coleoptile, the scutellar bundle forms part of the stele and turns downward within it (fig. 27). At this point two branches diverge from it which extend first laterally and then upward in the axis (figs. 37, 40). As they extend upward each branch in turn diverges. These last two traces thus derived, one on either side, are coleoptilar bundles (fig. 40). SARGANT and ARBER (18) describe the same situation by saying that half of each coleoptile bundle arises from the stele. Even though this is apparently true, the half coming from the stele comes from the same scutellar bundle that has turned downward within it. Longitudinal-face sections of the axis at this level make this unmistakable. The bundles from which the coleoptile traces are diverged extend upward, and from each, one or two (more often two) bundles diverge into the leaf above the coleoptile (fig. 40), and two or more into the second leaf above the coleoptile, etc. It should be pointed out that the scutellar bundle which turned downward in the stele at the level of the coleoptilar node, turns upward again at the scutellar node (figs. 27, 40). At this level a few vascular strands from the root add to it, and it extends upward in the stele, finally diverging as the midrib of the leaf above the coleoptile. The scutellar bundle, therefore, is entirely responsible for two (more often four) bundles of the leaf above the coleoptile, in addition to being partly responsible for its midrib. In a similar manner some of the traces of the second leaf above the coleoptile may be traced in their origin back to the scutellar bundle. Likewise the third leaf, etc.

In transverse section, the stele between the level of divergence of the scutellum and that of the coleoptile is characterized by two prominent endarch collateral bundles (figs. 46, 62). They are opposite, the protoxylem of each being toward the center of the stele. In origin they are one and the same bundle, and may be traced back to the scutellum (fig. 27). The central portion of the stele is often characterized by a large intercellular space. On either side of each collateral bundle are three or four groups of protoxylem and meta-

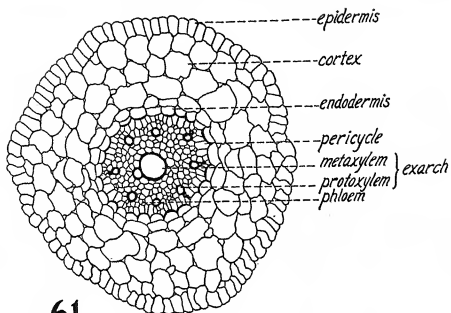


xylem vessels, some of which show varying degrees of exarch and endarch development. In the cortex just outside the stele and on the side of the axis toward the scutellum is the scutellar bundle previously described.

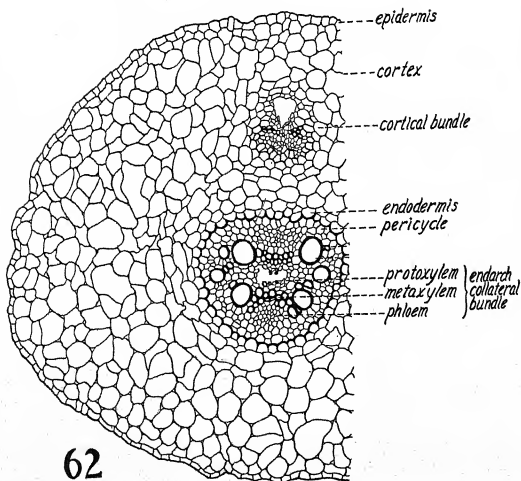
The internode between the coleoptile and the next foliage leaf is sheathed by the coleoptile (fig. 49). It differs anatomically from the higher internodes (fig. 63). It too is a "transition" interval, being more like the higher internodes than the internode next below it. It has a rather wide cortex, poorly organized vascular bundles, and an abundance of procambium with strands here and there differentiated into xylem and phloem. In structure the internode above the next leaf resembles more closely the higher internodes, although its bundles do not all have distinctly endarch xylem development (fig. 64).

**COLEOPTILE.**—The coleoptile is structurally similar to that of maize, although much smaller, being about the size of that of wheat (figs. 54, 55). Like the coleoptile of maize, it too is elevated by the growth of the elongating axis beneath it. The origin of its vascular system has already been described. The coleoptile of the oat differs from that of maize in the following respects: (1) the vascular bundles are small, with few xylem groups and relatively abundant phloem; (2) a bud is almost universally present in the axil of the coleoptile; (3) no more than the usual two vascular bundles have been observed in the coleoptile.

**LATER FOLIAGE LEAVES.**—Structurally the later foliage leaves of oat resemble more closely those of wheat (fig. 56) than of maize. Transverse sections show that the mesophyll is of much the same nature as in maize, the cells being closely arranged and with no well developed palisade layer. All the vascular bundles, except the midrib, are of essentially the same size. The mechanical tissue associated with the midrib is the chief support of the leaf. The bundle sheath cells are smaller than those of maize and do not possess chloroplasts. Usually their inner tangential walls are thickened. The motor cells are considerably less conspicuous. Frequently in both epidermal layers opposite the vascular bundles there are small, thick-walled sclerenchymatous cells similar to those found in the wheat leaf (fig. 56).

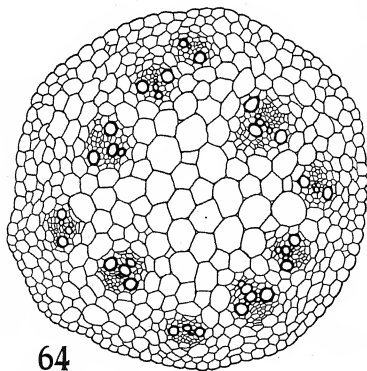
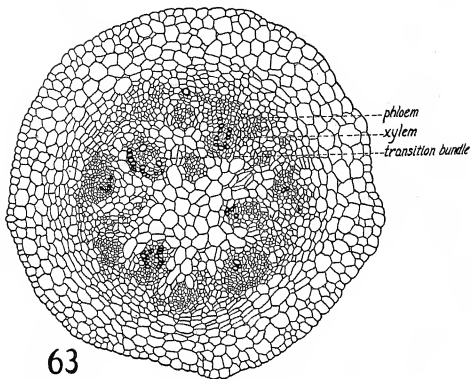


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FIGS. 61, 62.—*Avena sativa*: fig. 61, transverse section of primary root of seedling; fig. 62, transverse section of first internode of seedling.



FIGS. 63, 64.—*Avena sativa*: fig. 63, transverse section of second internode of 4-weeks-old plant; fig. 64, transverse section of third internode of 6-weeks-old plant.

## Wheat

### STRUCTURE OF EMBRYO

The orientation of the wheat embryo in relation to the caryopsis agrees with that of the oat embryo (fig. 5). In general appearance, likewise, the two embryos are similar (fig. 6). They both possess epiblasts, similar scutellar (fig. 9), and axial vascular systems (fig. 12); and in general a similar structural makeup. Some points of difference are: (1) The primordia of the seminal roots, excluding the primary root, occur in two pairs. They appear in the region of the scutellar node, being directed laterally downward. They may be seen in longitudinal-face section (fig. 12). (2) The scutellum is a short structure, extending only slightly beyond the tip of the coleoptile. Its ventral scale is therefore near its apex (fig. 6).

### GERMINATION

In general, the early stages of germination of the wheat embryo are comparable with those previously described in maize and oats. The coleorhiza first protrudes on germination after the rupture of the pericarp of the caryopsis. It is soon followed by the coleoptile (fig. 28). The root tip then digests and forces its way through the coleorhiza (fig. 29). Its appearance is closely followed by that of a pair of lateral roots, and a second pair appears in turn just above the first (figs. 30, 31). Inasmuch as the primordia of these lateral roots were present in the dormant embryo, they may be classed as seminal roots. In longitudinal-face section (fig. 12) the origin of these lateral roots may be traced to the region of the vascular plate of the scutellar node.

At about the time that the first pair of lateral roots emerges, the coleoptile begins to elongate and the leaf primordia within begin to swell. The coleoptile serves in early germination as a protection to the primordial leaves and stem as they push their way to the surface of the ground, always elongating just ahead of them. Slightly downward from the apex, on the side away from the scutellum, there is a minute opening in the coleoptile. The coleoptile, when traced in embryological development, is found to arise as a meristematic ring of cells on the lateral face of the embryonic mass of tissue which later differentiates into the scutellum. This ring of meristematic cells de-

velops into the characteristic conelike coleoptile. As the tip of the cone gradually becomes closed, the small pore just mentioned is left. The situation is essentially similar in maize and oats. During germination, as the tip of the coleoptile reaches the surface of the ground or shortly thereafter, the foliage leaf above the coleoptile pushes through this minute pore (fig. 31). The coleoptile may or may not come above the ground before its growth ceases, depending on the depth of planting and on environmental conditions. It may contain a trace of chlorophyll for a short period after its exposure to light, and stomata appear in its epidermis both above and below ground. Subsequent foliage leaves appear in alternate distichous arrangement, and the plant remains in this condition (with unelongated internodes) for some time (fig. 32).

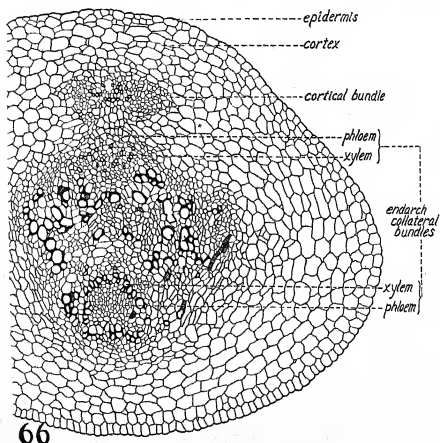
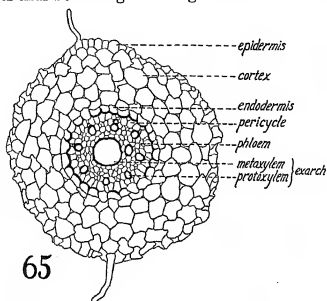
Until about two weeks after germination, no elongation of the internodes takes place except a slight elongation of the axis between the level of divergence of the scutellum and that of the coleoptile. After this period, the axis above the level of divergence of the coleoptile begins to elongate, elevating the nodes above it to approximately the surface of the ground (figs. 33-35). Throughout this time the elongating axis is sheathed by the coleoptile. A bud in the axil of the coleoptile now develops. Adventitious roots occasionally appear on the axis in the interval between the divergence of the scutellum and that of the coleoptile, and very often even above this latter level. PERCIVAL (16) contends that the adventitious roots appear in definite positions with respect to the leaves. He does not mention that roots occasionally appear at variable points in the interval between the level of divergence of the scutellum and that of the coleoptile.

#### ANATOMY OF SEEDLING

**SEMINAL ROOTS.**—The similarity in structure of the seminal roots of wheat and oats has been pointed out. A transverse section of the primary root of wheat may be seen in fig. 65.

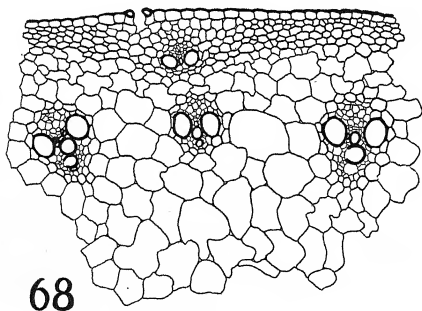
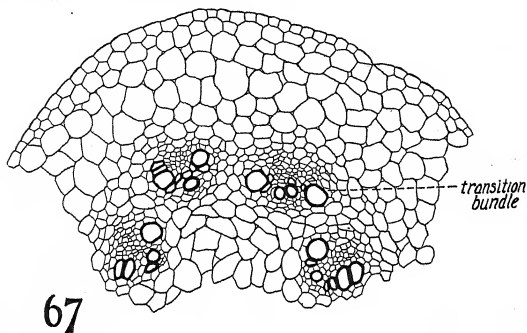
The adventitious roots appearing just above the level of divergence of the coleoptile are structurally similar to the seminal roots. They show several metaxylem vessels, however, instead of a large central one. The presence of more metaxylem vessels is correlated with that of a lesser amount of stelar parenchyma. This parenchyma

becomes lignified relatively early in adventitious roots, the protoxylem points thus becoming indistinguishable. Walls of the pericycle



FIGS. 65, 66.—*Triticum vulgare*: fig. 65, transverse section of primary root of seedling; fig. 66, transverse section of lower part of first internode of 4-weeks-old plant.

cells also become thickened. The number of protoxylem points varies from seven to eleven.



FIGS. 67, 68.—*Triticum vulgare*: fig. 67, transverse section of portion of second internode of 6-weeks-old plant; fig. 68, transverse section of portion of sixth internode of mature plant.

SEEDLING AXIS.—The first step in the "transition" from the exarch xylem arrangement in the root to the endarch condition char-

acteristic of the stem takes place in the region of the vascular plate at the level of divergence of the scutellum. Four seminal roots are initiated in this region, two on each side (fig. 12).

That part of the axis between the level of divergence of the scutellum and that of the coleoptile is very short. As previously pointed out, a slight elongation takes place in this region as the seedling develops (figs. 33-35). (The principal region of elongation in the young wheat axis is the internode above the coleoptile, as shown in fig. 35.) In the more pronounced cases of elongation, the vascular anatomy of the interval between scutellum and coleoptile is somewhat similar to the corresponding interval in the oat (fig. 62). In either case the stele in this region is largely a transitional structure. In the pronounced cases of elongation it shows in transverse section two colateral bundles. They are recognizable on opposite sides of the stele (figs. 47, 66). The arrangement of xylem, phloem, and parenchyma around them is very indefinite. Some xylem groups are exarch, some endarch, and others are indefinite. There is also a distinct bundle just outside the stele (fig. 66). With respect to this bundle the corresponding intervals on the wheat and oat axes are similar. The pericycle and endodermis are not recognizable with certainty. That part of the vascular skeleton which is derived at least in part from the scutellum, is markedly similar to that of the oat (figs. 38, 41).

The scutellar bundle within the axis diverges (frequently extending upward in the axis for a short distance before becoming part of the stele, as shown in fig. 35), the central third of it becoming part of the stele directly (fig. 41). Each outside divergence extends laterally upward for a short distance, then again diverges into two bundles, the outer one of each pair being a coleoptile trace. The inner bundle of each pair extends upward in the axis and finally diverges, giving rise to one or more bundles of the leaf above the coleoptile, as in the oat (fig. 40). The central third of the original scutellar bundle may be traced downward in the stele to the vascular plate at the scutellar node, where it turns upward. At this level a few vascular strands from the root add to it, and it extends upward in the stele, finally diverging as the midrib of the leaf above the coleoptile. The scutellar bundle is, therefore, entirely responsible for at least two bundles of the leaf above the coleoptile and partly re-



sponsible for its midrib. This is similar to the situation in oats. The vascular system of the coleoptile is, then, no more closely related to the scutellum than is that of the leaf above it. These facts would seem to indicate that the coleoptile bears the same relationship to the axis as the later leaves, except for the smaller number of coleoptile traces.

The internode between the coleoptile and the next foliage leaf (fig. 67) differs from the higher internodes of the axis (fig. 68) in about the same respects as the corresponding structures in maize and oats. Its bundles are not so sharply defined as are those of the higher internodes, and some transition between the exarch and the endarch xylem arrangement may be observed. In general, it more closely resembles the higher internodes than does that short interval in the axis between the level of divergence of the coleoptile and that of the scutellum. In contrast to the conditions in maize and oats, this internode is the principal elongating region of the seedling axis.

COLEOPTILE.—The origin of the vascular system of the coleoptile of wheat has been described. In transverse section its anatomy is so similar to that of the coleoptile of oats that no further description is necessary (figs. 54, 55). In contrast to the coleoptiles of maize and oats, however, that of wheat is not elevated (or only slightly so, as already pointed out) by a growth of the elongating axis, but instead, itself sheathes the elongating axis (figs. 35, 50). The coleoptile has been observed to reach a length of more than 4 inches. Its elongation depends on the depth of planting. PERCIVAL (17) reports the presence of from two to as many as six vascular bundles in the coleoptile of *Triticum dicoccum*. These bundles give the coleoptile distinct leaf-like characteristics, although it possesses no distinguishable midrib or blade.

LATER FOLIAGE LEAVES.—Structurally the leaf of wheat (fig. 56) is so similar to that of oats that no further description is necessary.

### Discussion

That the scutellum alone is the cotyledon of the Gramineae is highly probable in the light of this investigation. This opinion is based on evidence afforded by its relation to the embryonic axis, its vascular system, and its abundant parenchyma. Physiologically it

functions as a storage and absorptive organ. The scutellum will hereafter be considered the cotyledon. The so-called "ventral scale" of the cotyledon, when present, may be interpreted as the ligule of the cotyledon, as pointed out by STRASBURGER (24).

The principal opposing view, held by GOEBEL, SARGANT and ARBER, and others, is that the scutellum together with the coleoptile constitutes the cotyledon, the coleoptile representing the cotyledonary sheath. Such an interpretation is based partly on the consideration that the vascular system of the scutellum and that of the coleoptile are intimately related and are distinct from the vascular system of the parts of the plant above them. As GOEBEL (11) states: "the bundles of the coleoptile may be considered as branches of that which enters the scutellum." Or, according to SARGANT and ARBER (18): "the scutellum trace ceases to exist at the first node, but each of its two halves maintains its identity within one of the two coleoptile traces." It has been shown in the present paper that such is not the case.

In the seedling of *Avena*, for example, the scutellum bundle extends into the axis and continues upward in the cortex to the level of divergence of the coleoptile. At this level it forms part of the stele and turns downward within it. At this same level, two branches diverge from it which extend first laterally and then upward in the axis (figs. 37, 40). As they extend upward each branch in turn diverges, and the outer divergences, one on either side, are coleoptile traces (fig. 40). Some of the traces of the leaves distal to the coleoptile are derived from the diverged scutellum bundle in the same manner as are those of the coleoptile. In addition, the midrib of the leaf above the coleoptile originates in part from the scutellum bundle. In the seedling of *Zea mays* the coleoptile traces are also divergences from bundles that continue upward in the axis (fig. 39). The origin of the bundles from which the coleoptile traces diverge is too variable and too difficult to follow for any exact statement, although it seems certain that at least part of their vascular tissue can be traced to the scutellum. The situation in *Triticum* (fig. 41) is similar to that in *Avena*, although the interval in the axis between the scutellum and the coleoptile is usually not elongated. From this evidence it can be seen that the coleoptile traces bear no separate

and distinct vascular relationship to the scutellum. The scutellar bundle diverges, at least in part, into traces of two or more leaves distal to it. This evidence favors the interpretation that the coleoptile bears a similar vascular relationship to the axis that any of the early leaves above the cotyledon (scutellum) bear to it.

If the coleoptile were interpreted as being the cotyledonary sheath and the equivalent of a ligule or a pair of fused stipules, as has been held by WORSDELL and others, it would not be expected that the cotyledon would be borne at one level on the axis and its ligule or fused stipules at another, as occurs in *Avena* and *Zea*. The coleoptile should appear in the same relation that a ligule or a pair of stipules would bear to a foliage leaf, and be borne at the same node. As for WORSDELL's argument that the coleoptile is a ligule formed by the union of two stipules (based on the presence of the two vascular bundles which are commonly present in the coleoptile of *Zea mays*), I have previously shown that the coleoptile may possess from two to several bundles, thus indicating that it is not a double structure. There is no anatomical evidence that the coleoptile is a cotyledonary sheath, of either single or double nature.

This evidence, based particularly on the origin of the vascular supply and the anatomy of the coleoptile, would indicate that the coleoptile is the first independent leaf above the cotyledon, or the second leaf of the plant, and in consequence, not a cotyledonary sheath. Further support of this conclusion is given by the fact that buds are present in the axils of the coleoptiles of maize, oats, and wheat, which leaves little doubt as to the leaflike nature of the coleoptile.

It has been held that on the basis of origin the coleoptile must be part of the cotyledon. Such a conclusion might be based upon observations on the development of the embryo, because the coleoptile does arise from the lateral face of the embryonic tissue which later differentiates into the cotyledon.

This leads to the question of whether the cotyledon itself is a lateral or terminal structure. It is "terminal" if conclusions are drawn only from early embryogeny. Observations on the mature embryo, however, show the remains of the suspensor attached to the base of the coleorhiza (WEATHERWAX 28). This appears to be definite evidence

that the embryonic stem tip is terminal, and the cotyledon a lateral structure. The fact that the embryonic stem tip appears to arise from the lateral face of the immature cotyledon need not be construed as meaning that the cotyledon is terminal. A more logical explanation, in view of the position of the suspensor in the mature embryo, would be to consider the origin of the growing point of the stem as having been delayed in development until after the cotyledon had attained considerable size. In fact, in many dicotyledonous plants the cotyledons are often nearly mature before there is an indication of the presence of a growing point for the epicotyl, as for example, in *Capsella*. For these reasons, the cotyledon in grasses is interpreted as a lateral structure, and the growing point of the stem as a terminal one. The origin of the coleoptile is associated with the origin of the stem tip, and the coleoptile is, therefore, no more a part of the cotyledon than is the growing point of the stem itself. The coleoptile must, then, be interpreted in the light of its later development. The fact that the coleoptile is borne on the axis with a vascular relation to this axis similar to that of the later leaves makes it unlikely that it is part of the cotyledon.

The usual objections to interpreting the coleoptile as the second leaf of the plant are, first, that it is not alternate distichous with the cotyledon; and second, that it is not differentiated into sheath and blade as are the later leaves. These points do not seem to offer serious difficulties when one considers the number of cases in which the early leaves of a plant are entirely different from its later leaves, both in form and arrangement.

The epiblast is present in oats and wheat, but does not occur in maize. It has been interpreted by various investigators as a rudimentary second cotyledon. Such an interpretation is untenable because no vascular connection with the stele has ever been observed. The fact that it is not universally present in grasses, together with the fact that it has no vascular system, and arises from the coleorrhiza (as pointed out by CANNON and SOUÈGES), makes the epiblast appear to be of no more morphological significance than such a structure as the peg of the Cucurbitaceae. The conclusion of ARBER (1) and others that the epiblast bears essentially the same relation to the cotyledon that the auricles bear to the blade of the foliage leaf was

retracted (2) when she reiterated her earlier opinion (18) that it is of little significance.

The interval of the axis between the scutellum and the coleoptile, when obvious, has been variously interpreted: (1) as an elongated node between parts of a cotyledon (first named "mesocotyl" by ČELAKOVSKÝ); (2) as a unique structure that has resulted from a fusion of the cotyledonary stalk with the hypocotyl; and (3) merely as an internode.

The first interpretation can be dismissed because it is unlikely that the structure in question could be an elongated node in light of the present evidence which substantiates the view that the coleoptile is an independent leaf, the second of the plant.

The second view, held by SARGANT and ARBER (18), that the "mesocotyl" is neither a node nor an internode, but a unique structure such as described, may be of value in attempts to establish phylogenetic relationships between the grasses and some other monocotyledons, but other interpretations of it are equally plausible.

From the evidence in hand, there are two principal objections to the theory. First, if this interpretation be true, elongation of the embryonic axis would start in the same relative position in all types, regardless of the degree of fusion. As pointed out, however, this is not the case. The presence of the cortical bundle in the elongating axis of *Avena*, and its absence in *Zea*, result entirely from the specific location of the first meristematic region (figs. 18, 19, 25, 27). Second, such a fusion would not account for the vascular plate occurring at the level of divergence of the cotyledon in maize, oats, and wheat. This zone marks the cotyledonary plate anatomically, and it would be the first node of the plant as long as it exists, regardless of peculiar subsequent developments and of elongation in unusual places.

SARGANT and ARBER have further attempted to support their fusion theory by pointing out that the coleoptile bundles appear double and apparently have a double origin, that is, that one half of each arises from the cotyledonary bundle, continues upward in the coleoptile, then turns back on itself and continues downward in the stele. They illustrate the possible origin of this condition by diagramming an "imaginary type." There is no anatomical evidence that each coleoptile bundle is composed of two laterally fused bun-

dles; instead, the part apparently derived from the stele is of the same origin as that from the cotyledonary bundle, because the cotyledonary bundle simply turns downward within the stele at the level of divergence of the coleoptile. Each coleoptile bundle is therefore of single origin, and is not composed of two laterally fused bundles. This evidence confirms the statement of BUGNON (5):

Neither the direct observations of certain facts which would conform to the hypothesis (curve at the top of the coleoptilar bundles) nor the comparative anatomical study of lateral fusions of fibrovascular bundles, nor the ontogenetic study of cotyledonary development can permit us to accept the theory of the two English authors.

If the scutellum is the cotyledon and the coleoptile the second leaf of the plant, as the evidence in this study would indicate, then the third view, that the structure in question is an internode, is the correct one. According to this interpretation, the level of divergence of the cotyledon from the axis is the first node; that part of the axis immediately above the first node must of necessity be the first internode; the point of divergence of the coleoptile marks the second node; and the portion of the axis above this is the second internode.

Upon germination of the embryos of maize and oats, elongation of the axis begins in the first internode (figs. 19, 27). The second and subsequent internodes, sheathed by the coleoptile, are elevated to or near the surface of the soil. In the case of wheat, however, elongation is slight. The second node remains underground. The greatest elongation takes place in the second internode, elevating the third and subsequent nodes to the surface of the soil (fig. 35). The fact that the first internode in wheat often fails to elongate at all, in no way implies that it is not an internode.

The presence of the cortical bundle in the first internode of oats, and the fact that it first becomes part of the stele at the level of the coleoptilar node, have been construed as meaning that the vascular plate of the cotyledon is at the level of divergence of the coleoptile. As previously pointed out, there is a very definite vascular plate at the level of divergence of the cotyledon. The level of this cotyledonary plate remains the same regardless of later developments. The presence of the cortical bundle may be attributed entirely to the location of the first meristematic region of the axis (figs. 25-27).

The type of development of the xylem in the first and second internodes of maize, oats, and wheat has been pointed out. Whether or not any marked elongation of the first internode has taken place, both exarch and endarch xylem groups are present in it. The structure bears resemblance both to what is often referred to as typical root structure and stem structure. The second internode is more nearly typically stemlike, but still shows considerable transition in arrangement. This transition may continue even in the third and to some extent in the fourth internode. This condition of gradual transition through the lower internodes may be found in dicotyledonous plants, such as *Pisum* and others with hypogeal cotyledons. It may be regarded as supporting evidence in the interpretation that the interval in the axis between the level of divergence of the cotyledon and that of the coleoptile is the first internode, which displays a very markedly intermediate condition between root and stem structure.

### Summary

1. The scutellum in maize, oats, and wheat is the cotyledon. The "ventral scale" of the cotyledon, when present, may be interpreted as its ligule. The epiblast, when present, cannot be considered a rudimentary cotyledon; it probably has little morphological significance. These interpretations hold also for other grasses examined, including barley, rye, and rice.

2. The coleoptile is homologous with a foliage leaf, and is the second leaf of the plant, the leaf distal to it being the third leaf of the plant.

3. In maize and oat seedlings, the elongated structure between the cotyledon and the coleoptile is the first internode of the axis. The term "mesocotyl" as applied to this structure is meaningless. In wheat, the corresponding structure, although not elongated, is likewise the first internode of the axis. This holds true for other Gramineae that develop similarly to maize, such as oats and rice, and those that develop like wheat, such as barley and rye. The principal elongating structure in the young wheat axis is the second internode. It is always sheathed by the coleoptile.

4. There is no hypocotyledonary region of "transition" in the grasses. The transition is confined to stem structure. It starts and

takes place largely in the vascular plate at the first node, and in the first internode. The transition continues in the second internode, and to some degree even in the third and fourth internodes.

5. The three morphological types distinguished by VAN TIEGHEM are fundamentally one type, appearing differently upon development because of the difference in location of the meristematic region in the first internode. These differences often show in the mature embryo, before germination begins. The similarity of the vascular skeletons has been pointed out.

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#### LITERATURE CITED

1. ARBER, A., Leaves of Gramineae. BOT. GAZ. 76:374-388. 1923.
2. ———, Studies in the Gramineae. III. Outgrowths of the reproductive shoot, and their bearing on the significance of lodicule and epiblast. Ann. Botany 41:473-488. 1927.
3. AVERY, G. S. JR., Coleoptile of *Zea mays* and other grasses. BOT. GAZ. 86: 107-110. 1928.
4. BRUNS, E., Der Grasembryo. Flora 76:1-33. 1892.
5. BUGNON, P., La feuille chez les Graminées. Mém. Soc. Linnéenne de Normandie. 21:108. 1921.
6. CANNON, W. A., A morphological study of the flower and embryo of the wild oat, *Avena fatua* L. Proc. Calif. Acad. Sci. ser. III. Bot. 1:329-362. 1900.
7. CELAKOVSKÝ, L. J., Über die Homologien des Grasembryos. Bot. Zeit. 55: 141-174. 1897.
8. CLOS, D., Du collet dans les plantes, et de la nature de quelques tubercles. Ann. Sci. Nat. Bot. III. 13:5-20. 1849.
9. COULTER, J. M., The origin of monocotyledony. II. Monocotyledony in grasses. Ann. Mo. Bot. Gard. 2:175-183. 1915.
10. DEBARY, A., Comparative anatomy of the phanerogams and ferns (Eng. Transl. by F. O. BOWER and D. H. SCOTT). Oxford. 1884.
11. GOEBEL, K., Organography of plants. Part II (Eng. Transl. by I. B. BALFOUR). Oxford. 1905.



12. GRIS, A., Recherches anatomiques et physiologiques sur la germination. Ann. Sci. Nat. Bot. V. 15:70-83. 1864.
13. HEGELMAIER, F., Zur Entwicklungsgeschichte monokotyledoner Keime, nebst Bemerkungen über die Bildung der Samendeckel III. *Triticum vulgare* L. Bot. Zeit. 32:657-671. 1874.
14. DE JUSSIEU, ADRIEN, Sur les embryons monocotylédones. Ann. Sci. Nat. Bot. II. 11:341-361. 1839.
15. LESTIBOUDOIS, T., Phyllotaxie anatomique, ou recherches sur les causes organiques des diverses distributions des feuilles. Ann. Sci. Nat. Bot. III. 10:136-189. 1848.
16. PERCIVAL, J., The wheat plant. New York. 1922.
17. ———, The coleoptile bundles of Indo-Abyssinian emmer wheat (*Triticum dicoccum* Schübl.). Ann. Botany 41:101-105. 1927.
18. SARGANT, E., and ARBER, A., The comparative morphology of the embryo and seedling in the Gramineae. Ann. Botany 29:161-222. 1915.
19. SARGANT, E., and ROBERTSON, A., The anatomy of the scutellum in *Zea mays*. Ann. Botany 19:115-124. 1905.
20. SCHACHT, H., Lehrbuch der Anatomie und Physiologie der Gewächse. 1:323-334. 1859.
21. SCHLEIDEN, M. J., Sur la formation de l'ovule et l'origine de l'embryon dans les Phanérogames. Ann. Sci. Nat. Bot. II. 11:129-141. 1839.
22. SCHLICKUM, A., Morphologischer und anatomischer Vergleich der Kotyledonen und ersten Laubblätter der Keimpflanzen der Monokotylen. Bibliotheca Botanica Bd. 6. 1896.
23. SOUÈGES, R., Embryogénie des Graminées. Développement de l'embryon chez le *Poa annua* L. Compt. Rend. Acad. Sci. Paris 178:860-862. 1924.
24. STRASBURGER, E., Textbook of botany (Eng. Transl. by W. H. LANG). 5th Eng. ed. London. 1921.
25. TOOLE, E. H., The transformations and course of development of germinating maize. Amer. Jour. Bot. 11:325-350. 1924.
26. VAN TIEGHEM, P., Observations anatomiques sur le cotylédon des Graminées. Ann. Sci. Nat. Bot. V. 15:236-276. 1872.
27. ———, Morphologie de l'embryon et de la plantule chez les Graminées et les Cypéracées. Ann. Sci. Nat. Bot. VIII. 3:259-309. 1897.
28. WEATHERWAX, P., Position of scutellum and homology of coleoptile in maize. Bot. Gaz. 69:179-182. 1920.
29. ———, The story of the maize plant. Chicago. 1923.
30. WORSDELL, W. C., The morphology of the monocotyledonous embryo and of that of the grass in particular. Ann. Botany 30:509-524. 1916.

## RESPIRATION IN STRAWBERRY FRUITS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 399

ARTHUR R. GERHART

(WITH FOUR FIGURES)

### Introduction

Strawberry culture in the United States has become an important industry. In commercial value it has even taken rank with the white potato, as indicated in the United States Department of Agriculture Year Books. While Tennessee retains the leading position in strawberry culture, it is now followed closely by Louisiana. In Chicago the first shipments received come from Florida in December. By February the source of supply has shifted to Louisiana. From that time on until August there follow in rapid succession the producing areas of Mississippi, Arkansas, Tennessee, southern and northern Illinois, Wisconsin, Michigan, and even Montana as points of origin. Other consuming centers are of course supplied by a commerce equally complex.

Because the market and the point of production are often separated by great distances, and the fruits are highly perishable, the obstacles to commercial horticulture and market gardening (which are the causes of a really stupendous annual financial loss) are obviously of special severity in the strawberry industry. The present investigation was undertaken with the thought that it might help to throw light on the problems of transportation and storage, and at the same time have considerable purely scientific interest.

Botanical literature is replete with studies of the respiration of plant tissues. Most of these, however, deal with the seeds or with the vegetative parts, such as shoots and leaves of plants. Study of fruit respiration has been by comparison a neglected field. Also the usual procedure has been to measure the  $\text{CO}_2$  output rather than the  $\text{O}_2$  consumption, and to employ it as the sole index of respiration. Really, both  $\text{CO}_2$  and  $\text{O}_2$  have to be measured in order to arrive at

anything more than an approximate knowledge of the respiration process, as has been pointed out by HARRINGTON (16).

#### Source of material

The strawberries used were purchased in the Chicago market during the months from December, 1927, to July, 1928, and had been refrigerated to Chicago. The variety chiefly used was the Missionary, grown in both Florida and Louisiana. Some work was also done with the Gibson and the Brandywine. With regard to the effect of refrigeration on the respiratory rate, it may be stated at once that the present study is one concerned with strawberries that have undergone rapid transit and brief storage at temperatures of from 10° to 16° C.

Although fresh material was invariably secured from day to day, it was not noticed that a preservation of from two to three days in the ice-box had any noticeable effect upon the respiratory rate of a sound and uninjured berry. What the character of respiration of the freshly picked fruit is, this paper cannot attempt certainly to answer; although, from the results of some preliminary work with freshly collected berries in the autumn of 1927, it is suspected that it is not markedly different from the data here given. Nor are we dealing here with "normal" respiration which could only be determined by experimenting with undisturbed ripe fruits still attached to the plants.

Fresh-looking uninjured fruits were always selected from the boxes for use. The green calyx was not removed from the berry.

#### Apparatus

Since the KRAJNIK (19) respirometer has not previously been used in this country in work with plants, and only once, to the writer's knowledge, in experimental work of any kind (ALLEE 1, in his investigations upon isopods), a description of it (fig. 1) and its operation may be given here.

The apparatus consists essentially of a closed system of capillary tubing terminated at either end by a glass bottle. The bottle at the left contains the specimen being studied, while that at the right is empty and serves as a control. The advantage of a closed system, of course, is that changes of barometric pressure are obviated.

The middle of the tube is curved to form a loop and is set against a glass mirror bearing a millimeter scale. Into this part of the apparatus is introduced a column of kerosene colored with Sudan III.

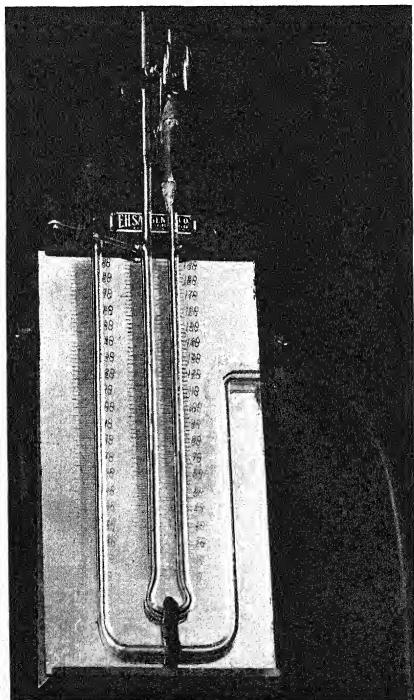


FIG. 1.—Krajičnik respirometer: tube containing kerosene in center; outer tube contains mercury.

This liquid moves up or down with reference to the scale in response to changes of gaseous pressure in the bulbs at the ends of the tube. In the apparatus as originally used by KRAJNÍK a single drop of liquid was employed instead of a column, as used in the present study. Connecting with the tube containing the colored kerosene on the left side of the system is a second capillary tube, which curves around below, forming a U, and which is made to contain mercury. The bottles are round-bottomed with a capacity of 75-80 cc. They are fitted with hollow ground glass stoppers and sealed with mercury (fig. 2). At a point on the inside surface of the glass stopper a small glass rod is attached which extends down into the bottle when the stopper is in place. At the lower end of this glass rod is a cup with a capacity of about 1 cc. to hold the carbon dioxide absorbent.

The entire apparatus is mounted on a wooden frame so that it may be placed in position over a constant temperature tank with the bottles completely submerged in the water. Two rubber connections and a single piece of glass tubing are necessary to connect each bottle to its end of the respirometer. These rubber connections

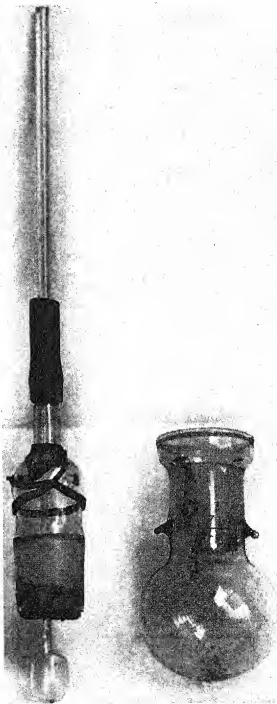


FIG. 2.—Container for respiring fruits

need not constitute more than a fraction of an inch in the length of the entire system, however, if the glass capillary tubes are just long enough to permit the proper submergence of the bottles. Moreover, as the  $\text{CO}_2$  was absorbed in the experiment, and it is not known that oxygen effects any very rapid union at low temperature with rubber, the use of thick-walled rubber tubing cannot be objected to.

The temperature of the water bath may be lowered by the use of ice, or raised by the heat from two large electric bulbs on a direct current. It may be maintained at any desired point by the use of a third bulb in a "make and break" circuit, with a well of mercury with which a platinum pin is adjusted to make contact at the desired temperature. The constant temperature tank used has inside dimensions of  $28'' \times 28'' \times 13''$ , and consequently holds a volume of water whose temperature is not readily changed by extraneous causes. Actually, modification of the temperature rarely amounted to as much as  $0.5^\circ \text{C}$ . during the course of any series of determinations, and was practically nil at  $25^\circ$  or above. In order to equalize the temperature of the water, it was kept constantly in motion by a stirring rod run by an electric motor.

### Operation

When, as a result of changes of pressure in one of the bottles, the kerosene moves either up or down with reference to the scale, it may be brought back to its original position by forcing the mercury farther up the vertical tube, or withdrawing it as the case may be, until the levels of the two columns of kerosene are back in their first positions. But this can only have been accomplished through a restoration of the original pressures. Since the volume of gas, and its temperature as well, in the right half of the system have not changed, the change in volume and hence of pressure must have been a result of the activities of the plant tissues confined in the bottle to the left. The amount of this volume change is then directly indicated from the scale set behind the mercury tube. By thus raising or lowering the mercury column, a manipulation readily accomplished by pressure of the fingers on the rubber tube attached to the free end of the mercury tube and which thus acts as a sort of bulb, readings may be successively made at any moment or interval

of time desired; or, at the close of each period of observation the stopcocks above (which admit air from the outside directly into the system) may be opened, thus restoring the pressure in the bulbs to that of the atmosphere, and closed again at the beginning of the next observation.

The advantage of this apparatus over all others known to the writer is its extreme sensitivity. It is possible with it to measure directly the respiration during a minute or even less time of one single strawberry.

For the purpose of greater clarity a single computation may be presented here. The diameter of the bore of the tubing is determined by careful measurement with a micrometer. From this the cross-section area is readily calculated and is found for the instrument used to be 0.02175 sq. cm. This value is used in all the computations, and is multiplied by the value in linear centimeters obtained from reading the instrument in order to calculate the volume change in cubic centimeters.

In one instance a strawberry weighing 4.2 gm. was used, the temperature of the water bath being 25° C. At 10:40:30" A.M., the level of the mercury being at 3 mm., the stopcocks above were closed simultaneously. The kerosene begins at once to rise in the left side of the instrument. Now the mercury is raised to counterbalance this change, and when the tops of the kerosene columns are again exactly level, the height of the mercury column is read and the exact time noted. The mercury is at the 42 mm. point and the time is 10:45. Then 4.2 cm.  $-0.3$ , or 3.9 cm.  $\times 0.02175 = 0.084825$  cc., the total volume of gas used. But the experiment has extended through four and one-half minutes; therefore  $0.084825 \div 4.5 = 0.019072$  cc.  $O_2$  was consumed in one minute. But the strawberry weighs 4.2 gm.; therefore  $0.019072 \div 4.2$  or 0.00454 cc. is the amount of oxygen consumed per gram of strawberry per minute of time, or 272.4 cc. per kilogram hour.

It was found by inserting a thermometer into a strawberry taken from the refrigerator, that several hours were required for it to reach room temperature; consequently berries intended for experiment were placed in an extra bottle and kept in the water bath for some time prior to their being placed in the respirometer for observa-

tion. It was discovered from blank trials that it was necessary to put the bottles in place and set the stirring rod in motion for about half an hour before observations were started, so that the temperature of the parts of the apparatus might be equalized. In order to facilitate this, the small cups after being filled with the 40 per cent NaOH solution (which was used to absorb the  $\text{CO}_2$  produced by the strawberries) were dipped for a moment into the water in the tank before being placed in the bottles. It was found of great importance to remove droplets of moisture which from any cause formed in the capillary tubing. It was likewise found that air currents set up in the room as the result of open doors and windows were to be avoided.

It may be well to point out that changes of volume of the gas in the bottle containing the berry cannot be determined practically by a direct reading of the change in the levels of the columns of kerosene. Were this attempted, a number of corrections to the values as obtained would be necessary. The first of these would be for the negative pressure in the gas in the control bottle. For as the kerosene moves from right to left a partial vacuum is set up in the control bottle. This would result in a reduced pressure, allowance for which would have to be made. Also, as the kerosene rises in one arm (the left) of the tube containing it, and falls in the other (the right), the weight of a column of kerosene whose height is the difference between the two levels acts as a negative pressure on the gas in the bottle containing the berry. Finally, it will be apparent that the loop of the capillary tube offers a considerable amount of friction to the passage of a tenuous column of liquid around it, and that once the loop is passed an accelerated movement will result, so that the rate of movement of the kerosene will be much faster during the latter part of a period than in the first part. By computation, it was found that the observed movement of kerosene corresponded to only one-tenth of the calculated change in volume when corrections were applied for the first two of these factors; and it would be difficult to calculate quantitatively the effect of the last one. Subsequent confirmation of the accuracy of the magnitude of this correction was afforded by the use of the proper method, and all of the difficulties and some others which need not be mentioned were avoided by its adoption.



## Results

OXYGEN CONSUMED IN RESPIRATION AT  
DIFFERENT TEMPERATURES

The first object was to measure initial rates of respiration of the strawberry at different temperatures. As it would not be feasible to include here all the data obtained, some series are presented as samples in table I. The table shows consumption of O<sub>2</sub>, the CO<sub>2</sub> being absorbed by NaOH solution, as previously mentioned.

TABLE I

AMOUNTS OF OXYGEN CONSUMED IN RESPIRATION AT DIFFERENT TEMPERATURES  
(MISSIONARY VARIETY)

| TEMPERATURE 5° C.,<br>WEIGHT OF BERRY 7.58 GM.      |  | TEMPERATURE 20° C.,<br>WEIGHT OF BERRY<br>6.60 GM. |  | TEMPERATURE 25° C.,<br>WEIGHT OF BERRY<br>5.856 GM.   |  | TEMPERATURE 36.5° C.,<br>WEIGHT OF BERRY<br>5.551 GM. |  |
|---|--|--|--|---|--|---|--|
| Duration of<br>experiment<br>(minutes)              | O <sub>2</sub> consumed<br>per gm.<br>per minute<br>(cmm.) | Duration of<br>experiment<br>(minutes)             | O <sub>2</sub> consumed<br>per gm.<br>per minute<br>(cmm.) | Duration of<br>experiment<br>(minutes<br>and seconds) | O <sub>2</sub> consumed<br>per gm.<br>per minute<br>(cmm.) | Duration of<br>experiment<br>(minutes)                | O <sub>2</sub> consumed<br>per gm.<br>per minute<br>(cmm.) |
| 38.....   | 0.7250   | 16.....  | 2.840  | 2:30...   | 4.434  | 6.....  | 8.290  |
| 24.....   | 0.7050   | 10.....  | 3.130  | 2:30...   | 4.434  | 8.....  | 9.207  |
| 18.....   | 0.6700   | 6.....   | 3.185  | 2:16...   | 4.508  | 6.....  | 8.881  |
| 51.....   | 0.7257   | 11.....  | 3.325  | 1:50...   | 4.238  | 7.....  | 8.956  |
| 15.....   | 0.6888   | 12.....  | 3.050  | 4: 0...   | 4.671  | 7.....  | 8.956  |
| 14.....   | 0.7173   | 18.....  | 2.966  | 10:10...  | 4.422  | 6.....  | 9.080  |
| 18.....   | 0.7010   | 18.....  | 2.929  | 15:10...  | 4.032  | 5.....  | 8.776  |
|   |  | 15.....  | 2.966  | 15:15...  | 4.344  | 7.....  | 9.120  |
|   |  | 20.....  | 3.000  | 15:10...  | 4.320  |   |  |
|   |  | 16.....  | 2.700  | 14:55...  | 4.380  |   |  |
|   |  | 10.....  | 2.768  | 9: 0...   | 4.166  |   |  |
|   |  | 14.....  | 2.871  | 11: 0...  | 4.220  |   |  |
|   |  |  |  | 12: 0...  | 4.240  |   |  |
|   |  |  |  | 12:45...  | 4.312  |   |  |
| Average O <sub>2</sub> consumed<br>per gm. per min. | 0.7132   | .....  | 2.977  | .....   | 4.337  | .....   | 8.908  |

The results obtained from Missionary berries are summarized in table II. All of these data are from observations made upon the berries at once after leaving them in the apparatus only long enough to attain the proper temperature ( $\frac{1}{2}$ – $2\frac{1}{2}$  hours). The figures therefore represent the initial respiration of berries at the temperatures indicated. The 191 trials made have an average length of 10–15 minutes.

Fig. 3 shows a respiratory curve constructed from these data. The significance of the figures when converted into market units may be seen readily. A box of strawberries of this variety as sold at the grocery weighs 661 gm. or 1.46 pounds, without the box. Eighty of these "quarts" are packed into a freezer or small refrigerator, of which about forty are packed into a car. At 20° C. (70° F.) these berries would consume 13.35 cubic feet of oxygen per hour; at 10° C. (52° F.) they would consume 5.34 cubic feet. If this oxygen were used in the oxidation of glucose, sufficient heat would be produced

TABLE II  
RATES OF RESPIRATION OF MISSIONARY STRAWBERRY

| TEMPERATURE<br>OF TEST (° C.) | CUBIC MILLIMETERS OF<br>O <sub>2</sub> CONSUMED PER GM.<br>OF BERRY PER<br>MINUTE | NUMBER OF<br>TRIALS |
|-------------------------------|---|---------------------|
| 5.....                        | 0.7132  | 7                   |
| 10.....                       | 1.1936  | 12                  |
| 15.....                       | 1.8000  | 12                  |
| 20.....                       | 2.9775  | 12                  |
| 25.....                       | 4.4920  | 74                  |
| 30.....                       | 6.3020  | 19                  |
| 35.....                       | 7.6796  | 18                  |
| 36.5.....                     | 8.6390  | 15                  |
| 37.5.....                     | 8.0430  | 8                   |
| 40.....                       | 4.7108  | 13                  |

to melt 318.7 pounds of ice in the first instance, and 127.5 pounds in the second.

In Louisiana, the berries are packed in pint boxes, weighing about 500 gm. or 1.1 pounds exclusive of the box. Twenty-four of these are put into a crate, of which about 800 are packed into a car. At 20° C. these berries would consume 64.87 cubic feet of oxygen per hour; at 10° C., 26 cubic feet per hour. The heat produced in these oxidations would melt 1548.5 pounds of ice in the first instance and 620 in the second.

From Tennessee come carloads of 420 cases of 24 quarts each. These also weigh about 500 gm. or 1.1 pounds. At 20° C., 31.78 cubic feet of oxygen would be required by them per hour; at 10° C., 12.7 cubic feet. The heat produced if these amounts of oxygen were used to oxidize glucose completely would melt 758 and 303 pounds of ice respectively.

## TEMPERATURE COEFFICIENT OF RESPIRATION

It may be seen from table II that the relations between the values for the respiration at  $5^{\circ}$  and  $15^{\circ}$ , that between those for  $10^{\circ}$  and  $20^{\circ}$ ,

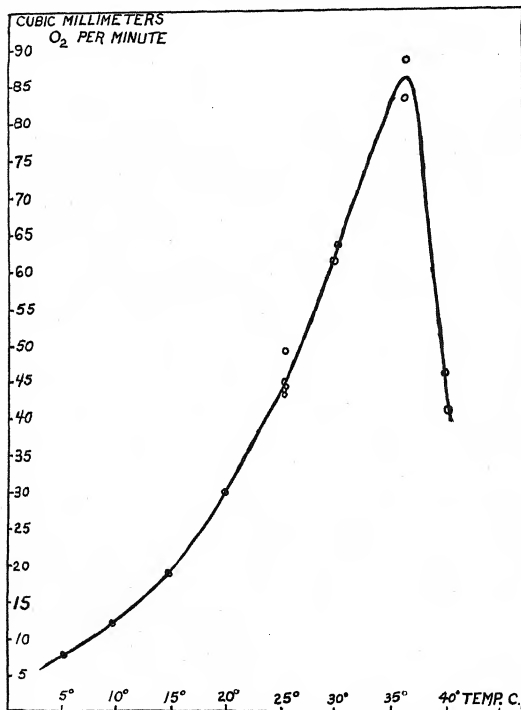


FIG. 3.—Graph showing respiration of Missionary strawberry

and also the relation between those for  $15^{\circ}$  and  $25^{\circ}$  are almost exactly (and within a very small error) what they would be assum-

ing a value of 2.5 for the  $Q_{10}$  of strawberry respiration. This result is quite in accord with the work of other students of respiration. CLAUSEN (9) found a  $Q_{10}$  value of 2.5 for wheat, lupine seedlings, and syringa flowers between  $0^{\circ}$  and  $20^{\circ}$ . GORE (15) found an average coefficient for a rise in temperature of  $2.377 \pm 0.024$  in his studies upon the respiration of fruits.

Above the  $25^{\circ}$  temperature, however, it is impossible to discover any consistent ratio in the data given (table II) for the strawberry. The reason for this is doubtless to be found in the fact that the cell membranes cannot be penetrated quickly enough by oxygen to prevent the formation of a deficit of this substance. This in itself would introduce a serious limiting factor to a continued geometrical rate of increase in the rate of a chemical reaction depending upon these materials. Further, when aerobic respiration becomes impossible, anaerobic respiration is initiated, resulting in the production of alcohol and various organic acids. These in turn, through their effect upon the protoplasm or their inhibiting effect upon the enzymes concerned, would introduce a further complication, so that it is impossible to discover any consistency with the Van't Hoff rule at the higher temperatures. It is not likely that the amounts of plastic materials available for oxidation would play the part of limiting factors in the case of respiration of fruits, as it might be expected to do, for example, with that of leaves.

CROZIER (10), in his efforts to identify the chemical processes in living matter, proposes to do so through the discovery of characteristic "critical temperature increments" which are identical in many different biological processes. The values which he finds (11500, 16100, and 16700 calories) have much the same significance as respiratory coefficients, in that they are expressions of regularity in the increase of the velocity of vital processes. He is compelled to assume a similarity between biological processes and a monomolecular reaction, which involves precisely the same obstacle to the successful application of the system of respiratory coefficients to rates of acceleration of biological respirations at higher temperatures.

#### MAXIMUM RESPIRATORY TEMPERATURE

It is common knowledge that plant activities are at their maximum rate at relatively high temperature. It is also known that from

the peak of activity maintained over a short interval of time the rate declines, although the temperature remains constant. Thus LEITCH (20), in studies upon the growth of *Pisum sativum*, recognized in addition to the customary absolute minimum, optimum, and maximum points, an additional fourth one, the maximum rate at a temperature point in which the time factor is likewise given consideration. BLACKMAN's (6) paper is also insistent on this point. Respiratory activity in the strawberry follows this rule. It will be seen from fig. 2 that the maximum rate of respiratory activity is in

TABLE III  
CUBIC MILLIMETERS O<sub>2</sub> CONSUMED PER MINUTE  
PER GM. OF STRAWBERRY

| TEMPERATURE<br>(° C.) |                          | NUMBER OF<br>TRIALS |
|-----------------------|--------------------------|---------------------|
| 30.....               | {Initial rate.....6.302  | 19                  |
|                       | {After 18 hours....5.304 | 4                   |
|                       | {After 24 hours....3.51  | 6                   |
| 35.....               | {Initial rate.....7.6796 | 18                  |
|                       | {After 18 hours....5.731 | 4                   |
| 36.5.....             | {Initial rate.....8.639  | 15                  |
|                       | {After 2 hours....7.921  | 7                   |
| 37.5.....             | {Initial rate.....8.043  | 8                   |
|                       | {After 2½ hours....7.688 | 9                   |
|                       | {After 3 hours....7.2    | 8                   |
|                       | {After 6 hours....6.8836 | 7                   |
| 40.....               | {Initial rate.....4.7108 | 13                  |
|                       | {After 22 hours....3.25  | 12                  |

the neighborhood of 35.5° C. But this rate is not maintained for very long, very quickly falling. Thus Missionary strawberries whose initial rate of respiration at 30° C., as already shown, is 6.302 cu. mm. per minute, becomes 5.305 in 18 hours' time and 3.51 at the end of 24 hours.

The rate established as the initial one at 35° falls more quickly in 18 hours from 7.679 to 5.731. While that discovered for the 36.5° (8.639) falls in two hours to 7.921. The rate for 37.5° (8.0) drops in 2.5 hours to 7.688, in 3 hours to 7.2, and in 6 hours it has become 6.8836. The rate at 40° (4.4119) likewise falls to 3.2519 in 22 hours. These results are summarized in table III, and expressed graphically in fig. 4.

It may be seen that the higher the initial rate the more rapidly it falls. The highest two rates (that is, those for  $36.5^{\circ}$  and  $37.5^{\circ}$ ) at the expiration of three hours are less than the rate for  $35^{\circ}$ , and the rates for all the temperatures employed above  $25^{\circ}$  show a disposition to fall below the initial  $25^{\circ}$  rate, which was approximately 4.45 cu. mm. per minute (table I). Thus it appears that over a period of 6 or 8 hours the optimum rate would not be at  $36.5^{\circ}$  nor yet at  $37.5^{\circ}$ ,

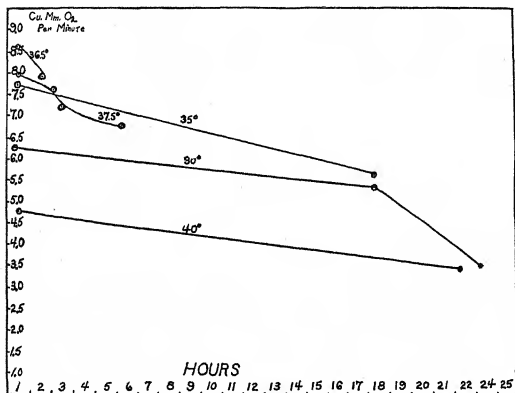


FIG. 4.—Curve of respiratory rate at temperatures indicated over periods of time (Missionary).

but rather at  $35^{\circ}$  and for longer periods it would be even lower. Thus accepting LERTCH's definition of optimum as the temperature in which no time factor enters, it would probably be between  $25^{\circ}$  and  $30^{\circ}$  C.

The strawberry appears to suffer injury at higher temperatures. It is for this reason difficult to secure readings of the respiratory rate upon berries which appear at the close of the series of trials to be in normal condition.

## HUMIDITY FACTOR IN RESPIRATION

In the major part of the work here reported, no attention was paid to the factor of humidity as influencing respiration. What possible effect it might have was shown by the following experiment.

The work was done with the Gibson strawberry, grown in Wisconsin. Respiration rate was first determined for the berries as they were obtained on the market. The results of two series of determinations were as follows: first series of seven trials, aggregating 125 minutes, 2.505 cu. mm. of oxygen per minute; second series of six trials, aggregating 123 minutes, 2.508 cu. mm. of oxygen per minute. The readings were unusually consistent throughout.

Two lots of these berries were then placed in desiccators, the one containing  $\text{CaCl}_2$ , the other a beaker containing water. The desiccators were allowed to remain at the temperature of the laboratory for 24 hours. At the close of this period, a series of readings was made of the respiration of the berries in the  $\text{CaCl}_2$  desiccator. In this experiment, as with the one later described, of the berries set in the  $\text{CaCl}_2$  desiccator,  $\text{CaCl}_2$  was also put in the bottle in which the berry respired while under observation. The results of eleven series of observations, extending over 184 minutes, gave an average value of 2.780 cu. mm. of  $\text{O}_2$  consumed per minute per gram of berry.

At the expiration of 48 hours respiratory activity was again observed. The strawberries in the  $\text{CaCl}_2$  desiccator, as the results of seven tests of 123 minutes in duration, were shown to be respiring at the rate of 1.885 cu. mm. of  $\text{O}_2$  per minute per gm. of berry, while those from the humid chamber were using 1.992 cu. mm. These latter results were obtained as a result of five measurements of 70 minutes' duration. These results are presented in table IV.

It appears from the figures that a temporary increase in the rate of respiration occurred as a result of the stimulus of dry air, and that this later fell off to a point slightly below that of the berries held in moist air. This is in accord with the work of LUTHRA (22) upon Conference pears. The results seem to justify the assumption that ordinary fluctuations of atmospheric humidity can be disregarded to a considerable extent as a factor affecting rates of respiration in the strawberry.

In a further effort to substantiate this conclusion, a determina-

tion was made of the actual water content within the berry after an exposure to dry air. The first method was that of placing weighed quantities of berries in the desiccator with  $\text{CaCl}_2$ , and contrasting their loss of weight with that of berries held in a humid chamber. Berries used were carefully selected for soundness, with no abrasions of the epidermis. Table V gives the results of these tests.

TABLE IV

EFFECT OF HUMIDITY UPON RESPIRATORY RATE OF GIBSON STRAWBERRY  
(ALL MEASUREMENTS AT 20° C.)

| CONDITIONS   | RESPIRATORY RATE<br>EXPRESSED IN<br>CMM. OF $\text{O}_2$ CON-<br>SUMED PER GM. OF<br>BERRY PER MIN. | NUMBER OF<br>TRIALS | TIME IN<br>MINUTES |
|--|---|---------------------|--------------------|
| Newly purchased berries:   |   |                     |                    |
| First series . . . . .   | 2.505   | 7                   | 125                |
| Second series . . . . .  | 2.508   | 6                   | 123                |
| After 24 hours in desiccator con-<br>taining $\text{CaCl}_2$ . . . . . | 2.780   | 11                  | 184                |
| After 48 hours in desiccator con-<br>taining $\text{CaCl}_2$ . . . . . | 1.885   | 7                   | 123                |
| After 48 hours in moist chamber.                                       | 1.992   | 5                   | 70                 |

TABLE V

LOSS OF WEIGHT OF MISSIONARY STRAWBERRIES IN DRY AND MOIST AIR

| CONDITIONS   | ORIGINAL<br>WEIGHT<br>OF BER-<br>RIES<br>(GM.) | WEIGHT<br>AFTER<br>17 HOURS<br>(GM.) | AMOUNT<br>OF LOSS<br>IN<br>WEIGHT<br>(GM.) | PERCENT-<br>AGE LOSS<br>IN<br>WEIGHT | WEIGHT<br>AFTER 50<br>HOURS<br>(GM.) | TOTAL<br>LOSS IN<br>WEIGHT<br>(GM.) | PERCENT-<br>AGE OF<br>LOSS |
|--|--|--------------------------------------|--|--------------------------------------|--------------------------------------|-------------------------------------|----------------------------|
| Placed in desiccator con-<br>taining $\text{CaCl}_2$ . . . . . | 80.60  | 80.04                                | 0.56                                       | 0.695                                | 79.03                                | 1.57                                | 1.95                       |
| Placed in humid chamber  | 79.16  | 78.86                                | 0.30                                       | 0.380                                | 78.28                                | 0.88                                | 1.11                       |
| Difference . . . . .   |  |                                      |  | 0.315                                |                                      |                                     | 0.84                       |

Only those results obtained after the lapse of considerable time, for example, 17 hours, are regarded as especially significant, in as much as the slight difference between the weights at earlier periods might well be due to a loss of hygroscopic moisture from the surfaces of the berries. From these results, it appears that the epidermis of a



strawberry must be an exceedingly effective covering for the prevention of water loss. Therefore, insofar as dry air acting upon respiration of strawberries indirectly (by causing an undue water loss) is concerned, its effect is demonstrably small.

This decline in weight is not to be thought of as the rate at which boxes and crates of strawberries on the market lose weight. The decline is in reality considerably faster, owing to the presence generally of many diseased and injured fruits. This fact the following measurements will show:

#### FIRST MEASUREMENT

A "pint" box (box weighing 50 gm.) of Gibson strawberries was used just as received at grocery.

| WEIGHT   | GM.    |
|--|--------|
| Including box, Thursday, July 5, 5:00 P.M.           | 555.12 |
| After storage in icebox, Friday, July 6, 3:20 P.M.   | 549.95 |
| After storage in icebox, Saturday, July 7, 9:00 P.M. | 534.00 |
| After storage in icebox, Monday, July 9, 4:30 P.M.   | 505.00 |

Thus, in 4 days' time the strawberries had lost 10 per cent of their weight. At this time the berries were moldy and in bad shape. Upon picking them over, only 176 gm. of sound berries remained from the entire lot.

#### SECOND MEASUREMENT

A "quart" box of Florida-grown Missionary strawberries when received at the grocery (including the box in which they were packed, weighing 42 gm.) was used in these measurements, taken after storage at 10° C.:

| WEIGHT                         | GM.   |
|--------------------------------|-------|
| Thursday, March 15, 2:00 P.M.  | 703.0 |
| Friday, March 16, 2:45 P.M.    | 681.0 |
| Saturday, March 17, 2:00 P.M.  | 664.0 |
| Monday, March 19, 11:00 A.M.   | 631.3 |
| Tuesday, March 20, 2:00 P.M.   | 612.0 |
| Wednesday, March 21, 1:00 P.M. | 594.5 |

Again, in 4 days' time the berries had lost 10 per cent of their weight. At this time the moldy and decayed berries were removed, leaving but 214.3 gm. of sound ones.

A still further confirmation was sought by determining the percentage of moisture contained in strawberries as a result of exposure to desiccated air. The results are shown in table VI.

The data obtained in these tests may be compared with that of an analysis made upon berries as they arrived on the market. The duplicates showed 89.67 and 89.89 per cent of water loss, or an average of 89.78 per cent.

These results confirm the conclusion that water loss is very little greater in dry than in moist air, and that consequently the indirect effect upon respiration as a result of such water loss cannot be great. This conclusion is not vitiated by the results of KOLKWITZ (29),

TABLE VI  
MOISTURE DETERMINATIONS WITH GIBSON STRAWBERRY AFTER  
RESPIRING IN MOIST AND DRY AIR

| WEIGHT   | FIRST SAMPLE<br>(GM.) | SECOND SAMPLE<br>(GM.) | AVERAGE<br>(PER CENT) |
|--|-----------------------|------------------------|-----------------------|
| After respiring 24 hours in desiccator.....        | 14.870                | 14.470                 |                       |
| After drying 12 hours in vacuum oven at 60° C..... | 1.608                 | 1.5330                 |                       |
| Loss in weight as result of drying                 | 13.262                | 12.9370                |                       |
| Percentage loss.....                               | 89.186                | 89.4050                | 89.2955               |
| After respiring 24 hours in moist chamber.....     | 17.480                | 17.5000                |                       |
| After drying 12 hours in vacuum oven at 60° C..... | 1.780                 | 1.7312                 |                       |
| Loss in weight as result of drying                 | 15.700                | 15.7688                |                       |
| Percentage of loss.....                            | 89.817                | 90.1670                | 89.9626               |
| Difference in moisture content.....                |                       |                        | 0.6671                |

DUVEL (12), BAILEY and GURJAR (4, 5), and many others, who find that moisture accelerates respiration in seeds. In the respiration of seeds, moisture is doubtless a limiting factor.

Although over long periods of time considerable metabolic water is produced as a result of aerobic respiration, as BABCOCK (3) has shown, the amount of water contained in the plant tissues appears to be more or less carefully regulated. Thus MORSE (25), working with apples, finds a constant proportion of water and dry matter which existed over a length of time of several months, in spite of a continuous loss of water and decrease of weight. A dry weight determination made upon green strawberries showed percentages of moisture of 90.44 and 90.61 in the duplicates, the average being

90.525. This somewhat surprising result, showing a moisture content equal to that in the ripe berries, also the results upon ripe strawberries just given, together with the results of other analyses,<sup>1</sup> indicate not only that this condition of water equilibrium is maintained in the strawberry as in the apple, but also suggest that approximately the same amount of water exists in the fruit over the entire ripening period. In this connection, the findings of LOREE (21) are of interest. Experimenting with various fertilizer treatments of strawberries, he found that although the size of the berries, number of flower clusters, and nitrogen and sugar content are subject to variation, very little effect is noticeable on the moisture content of the fruit. Accordingly, save for a temporary effect of perfectly dry air in stimulating respiration, an influence which would not be likely to exist in practical marketing operations, the entire matter of water within and moisture without the berry ceases to be really significant in respiration. This fact, however, does not mean that humidity may not be an important factor in marketing losses from disease organisms or other types of physiological behavior.

#### RESPIRATION OF GIBSON STRAWBERRY

The only study upon the respiration of the strawberry which is available in the literature is that of GORE (15). His curve for the respiration of the Gandy variety is based upon three determinations. His results, translated into the units employed in this paper, are as follows:

|               |        |  |
|---------------|--------|--|
| 2° C. ....    | 0.1283 | cu. mm. CO <sub>2</sub> per gm. berry per minute |
| 10.6° C. .... | 0.405  | " " " " " " " "                                  |
| 26.2° C. .... | 1.405  | " " " " " " " "                                  |

His respiration curve for Martin's New Queen's strawberry is determined by two measurements:

|               |      |  |
|---------------|------|--|
| 10.1° C. .... | 0.36 | cu. mm. CO <sub>2</sub> per gm. berry per minute |
| 23.9° C. .... | 1.20 | " " " " " " " "                                  |

<sup>1</sup> LOREE, as a result of 54 determinations of water content on ripe strawberries of different varieties, found values ranging from 87.9 to 92.8 per cent. In a series of analyses undertaken primarily to show loss of dry weight as a result of respiration, the averages of the two samples showed as a result of the combined effect of water removal and loss of dry weight due to respiration, a loss of 89.78 per cent after 48 hours at 25° C., 91.25 per cent after 72 hours, and 91.56 per cent after 96 hours.

These two rates of respiration are closely similar. They are strikingly different, however, for the rate of respiration of the Missionary variety as previously shown here, the difference being roughly 200 per cent for the rates at about the 10° and 25° temperatures.

In a series of experiments conducted with the Gibson variety, a much higher rate even than that for the Missionary was shown to obtain at the lower temperatures. But the rate for the Gibson at 20° is not much more than half that for the Missionary, and is lower than that for its own 15° rate (table VII).

TABLE VII  
RESPIRATION OF GIBSON STRAWBERRY

| TEMPERATURE<br>(° C.) | O <sub>2</sub> CONSUMED<br>PER GM. BERRY<br>PER MINUTE<br>(CU. MM.) | NO. OF<br>TRIALS |
|-----------------------|---|------------------|
| 5.....                | 0.7961  | 6                |
| 10.....               | 1.4750  | 14               |
| 15.....               | 2.7116  | 19               |
| 20.....               | 2.5065  | 13               |

The point which it is desired to emphasize is that there is an enormous disparity between rates of respiration of different varieties of strawberries. It should be mentioned in this connection that the Missionary strawberries were grown in Florida and the Gibson in Wisconsin. GORE does not state the place of origin of his fruit. The facts that the strawberries of one variety (which respire at a maximum rate at a high temperature) were grown in a region of high temperatures, and those of another variety were grown in a region of low temperatures (and respire most rapidly at comparatively low temperatures) may be a correlation; it is not in any sense a physiological explanation of the difference.

An interesting parallel to this is found in the studies of rates of respiration made upon apples. DRAIN (11) states that apple varieties vary greatly in respiration rates, and his conclusions are certainly borne out by his results, the variations being from 300 to 400 per cent, and in some instances from 600 to 800 per cent between respiratory rates of Oldenburg and Maiden Blush apples at the same temperatures. MORSE (25) also finds that the Oldenburg respire

twice as much at 25° as the Winesap. DE VILLIERS (32) likewise finds respiration of grapes varying with varietal differences.

These striking contrasts bring into sharp relief the character of the obstacles to an understanding of the nature of respiration. Specific characters of plants are certainly not a guide. It is not the purpose here to present even a summary of the variety of influences which have at one time or another been advanced as "controlling" ones, much less the still larger number of contributing factors. Among the more recent suggestions, however, the one by WILLAMAN and BEAUMONT (33) may be noted. Conclusive data are presented to show that respiration is depressed in an atmosphere of CO<sub>2</sub>, but that respiration, as measured by CO<sub>2</sub> output, is renewed with great vigor when the tissues under observation are "aspirated." WILLAMAN and BEAUMONT reject the simplest explanation of this behavior, to wit, that an equilibrium between the CO<sub>2</sub> of the tissues and that of the atmosphere is being reached. They advance the hypothesis that CO<sub>2</sub>, by increasing the hydrogen-ion concentration, brings the proteins of the protoplasm to their isoelectric point, thus rendering them more permeable. To the objection that such increase in permeability would operate to increase CO<sub>2</sub> production immediately, they suggest that the increase in permeability would probably be merely potential so long as there was a high content of CO<sub>2</sub> surrounding the tissues, but would become actual as soon as aspiration commenced. No measurement of O<sub>2</sub> consumption is reported in their study.

Numerous attempts have been made to correlate respiratory activity with the presence of catalase. Such a correlation has been found by APPLEMAN (2), SHERMAN (30), STEHLE (31), and others. RHINE (28) found no correlation between respiration and catalase activity in the early stages of seed germination, and thought the connection if any was indirect. The results of three attempts to measure catalase in the strawberry pulp led to the liberation of an amount of O<sub>2</sub> so small as to be difficult of measurement, although strawberry fruits respire actively.

GORE (15) states some of the further perplexities in his study of respiration of fruits: "No satisfactory theory based on the composition or size of fruits has been found to account for the differences in

the respiratory activity . . . the rate of respiration is not a direct function of content of sugar or of acid and does not depend upon size." (Not correlated with surface area as against mass?)

PALLADIN'S (26) disposition to divide respiration into several different categories, each with its distinct causal agent, as oxidase, nucleo-proteins, protoplasm, etc., and to separate the process into different stages is, it would seem, a helpful concept.

#### RESPIRATORY RATIO

As a result of about twelve different experiments on as many strawberries, extending over about 60 hours' time, it can be stated definitely that the ratio  $\text{CO}_2/\text{O}_2$  is greater than one in strawberry respiration. This generalization holds for both green and ripe fruits. The determinations were made by placing the strawberries in the Krajnik apparatus without the NaOH solution to absorb the  $\text{CO}_2$  produced. It was necessary to remove the green calyx from the fruits so as to avoid errors as a result of photosynthesis.

It is clear that any changes in the levels of the kerosene under these circumstances will be the result of a difference between the volume of  $\text{O}_2$  consumed and the volume of  $\text{CO}_2$  released. In all cases the kerosene was depressed in the left side of the tube. The method of measurement, therefore, requires the raising of the mercury to a high point in its tube at the beginning of the experiment, and then restoring the unequal levels of the kerosene columns by lowering it at the end; in short, just the reverse of the manipulation for measuring the oxygen consumption.

Owing to the fact that the movement of kerosene is very much slower than when the entire amount of one of the gases is being measured, the possibility of error is very much greater than in the measurements of  $\text{O}_2$  consumption. The value of 1.2 for the respiratory ratio will have to be submitted as an approximation at the present time. The existence of a high respiratory ratio may be associated with production of alcohol and acids, as in the case of anaerobic respiration.

Finally, such a ratio can be interpreted as indicating an oxidation of tannins (CHATIN 8), and more especially of organic acids (GERBER 13).

A decline in acid content seems to be general in the ripening and during the storage of many fruits. For example, MAGNESS (24), although he reports a coefficient of respiration of not more than one, finds a consistent decrease in acidity with apples in storage at 32° F.<sup>1</sup> Results of some analyses of the acid content of the strawberry show this to be the case with this fruit. In all cases the results are averages of duplicate samples, and are expressed in number of cc. N/10 NaOH necessary to neutralize the acid content of 1 gm. of strawberry. Samples consisted of from 10 to 35 gm. of fruit.

TABLE VIII  
ACID ANALYSES OF STRAWBERRIES

| CONDITION                               | FIRST LOT<br>(cc.) | SECOND LOT<br>(cc.) |
|---|--------------------|---------------------|
| Green.....                              | 2.78               |                     |
| Partly green.....                       |                    | 3.02                |
| Early ripe.....                         |                    | 2.39                |
| Average market condition<br>(ripe)..... | 2.6                | .....               |
| After standing                          |                    |                     |
| 24 hours at 25°.....                    | 2.54               | .....               |
| 24 hours at 14°.....                    |                    | 2.03                |
| After standing                          |                    |                     |
| 48 hours at 25°.....                    | 2.58               | .....               |
| 48 hours at 14°.....                    |                    | 2.07                |

From this it appears that a decline takes place in the acidity up to the time that the berries are fully ripe and have reached a stage that may be described as over-ripe.

In the present state of knowledge, there is nothing to prohibit the use of the hypothesis proposed by GERBER that the oxidation of organic acids during respiration supplies an explanation of the high respiratory ratio of the strawberry.

#### EFFECT OF ETHYLENE GAS ON RESPIRATION

A topic of interest in the current literature in plant physiology is the effect of ethylene gas and other stimulating chemicals in the

<sup>1</sup> The production of "disagreeable flavors" in apples noted by MAGNESS (24) as a result of various methods of excluding oxygen, and the "fermented flavor" in cherries and the "very bad flavor" of peaches under similar conditions described by HILL (18) are in accord with common experience in the handling of fruits. A point to be emphasized, however, is that a loss of palatability must not be confused with increased acidity.

ripening processes of fruits and vegetables, and concurrently its relation to respiration (CHACE and DENNY 7, MACK 23, HARVEY 17, REGEIMBAL, VACHA, and HARVEY 27). Does ethylene accelerate the natural process of ripening of bananas, lemons, tomatoes, cantaloupes, etc., with its attendant increase of sugar content and juiciness, or are the heightened colors produced by it due to the occurrence of what may be described as chemical artifacts unrelated to normal maturation?

In an endeavor to secure an answer to this question, several shipments of green Missionary strawberries were ordered from Plant City, Florida, in April (1928) to be sent via air mail and special delivery. The fruit arrived in good fresh condition. Two constant temperature ovens, of a capacity of 102.56 liters, after being carefully sealed with plasticine were used as gas-tight chambers for the experiment, one oven being used for the controls. In one instance about a pint of berries were placed in the oven with ordinary dry air. In a second experiment, the berries in the first instance having shown signs of withering, a number of glasses of water were inclosed with them. In a third trial, two lots of green strawberries were placed in inverted wide-mouthed bottles, the corks of which were protected by tinfoil and penetrated by small pieces of glass tubing, on the lower ends of which were short pieces of rubber tubing clamped by pinch cocks. The temperature was that of the room, about 20° C. Ethylene gas was measured in a gas manometer, and then forced into the oven by a leveling tube filled with mercury. The ethylene was introduced into the bottle in a small section of ordinary glass tubing whose internal capacity had been determined carefully. This glass tube was filled with ethylene by displacement under mercury. It was then connected to the wide-mouthed bottle containing the strawberries, the pinch-cock released, and the gas which it contained allowed to diffuse into the bottle. In the first experiment, doses of 1 part of ethylene to 1000 parts of air were introduced. In the second experiment the dose was cut down to 1 part in 2000. The dosage for the experiment in the bottle was about 1 part in 1500. Ten doses were given in the first experiment, distributed over three days. Eight doses were used in the second experi-



ment with the oven, and also in the third experiment with the bottle, both of which lasted the same length of time.

No results of the effect of ethylene gas could be recognized in any of the experiments. Seen in different lights, spread out, turned over, rearranged, inspected carefully by a number of individuals called to aid, no differences from the controls in redness, yellowness, hardness, juiciness, or taste could be distinguished at all. The direct effect of ethylene on respiration was not measured.

### Summary

1. An exceedingly sensitive respirometer is described, by which the rate of respiration of a single strawberry over half a minute of time can easily be measured. The method of operation is explained. Large sources of error inherent in an impracticable method of use of the instrument, but one which plausibly suggests itself, are pointed out.

2. The amount of oxygen used by the Missionary strawberry at temperatures of from 5° to 40° C. is determined. A curve of this respiratory activity is presented.

3. Amounts of oxygen required by strawberries during shipment in carload lots is calculated. Assuming that the oxygen so required is used to oxidize glucose, the amount of heat thereby generated is shown in terms of pounds of ice which it would melt.

4. The maximum initial rate at which strawberries respire is 36.5° C. At the higher temperatures (35°, 36.5°, 37.5°, and 40° C.) respiration quickly falls. As a result, the maximum rate varies with the time over which it is considered.

5. The temperature coefficient for the respiration of the Missionary strawberry is 2.5 for temperatures below 25° C.

6. A temporary increase in the rate of strawberry respiration occurs as a result of exposure to dry air. This rate later declines.

7. Strawberries are very effectively protected from water loss in dry air. The loss is about 2 per cent by weight at the end of 50 hours in dry air, compared with a loss of 1.1 per cent in moist air.

8. Strawberries have a very constant water content, the amount of which is approximately 89.5 to 90 per cent.

9. Deterioration of strawberries in storage at ice-box temperatures as measured by loss in weight is about 4.5 to 5 per cent in two days and 10 per cent in four days.

10. The Gibson strawberry, northern grown, has a much higher rate of respiration at low temperatures than the Missionary strawberry, which is southern grown.

11. Much of the data available upon respiration is inconsistent with the interpretations made of it. This is because of inability so far to reckon in given instances with all the factors affecting respiration.

12. Acid content of strawberries declines as they pass from the green stage to a stage of ripeness just preceding actual deterioration.

13. The respiratory ratio ( $\text{CO}_2/\text{O}_2$ ) for ripe fruits is found to be greater than one. An approximate value of 1.2 is given for this ratio. The  $\text{CO}_2/\text{O}_2$  ratio was determined with the Krajnik apparatus.

14. Ethylene gas in concentrations of 1:1000, 1:1500, and 1:2000 is without effect in hastening the visible changes incident to ripening in strawberries.

The problem here considered was suggested by Professor CHAS. A. SHULL of the Hull Botanical Laboratory of the University of Chicago. To him, and to Dr. SCOTT V. EATON, thanks are due for suggestions and aid in many ways.

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#### LITERATURE CITED

1. ALLEE, W. C., Studies in animal aggregations: causes and effects of bunching in land isopods. *Jour. Exp. Zool.* 45:255-277. 1926.
2. APPLEMAN, C. O., Some observations on catalase. *BOT. GAZ.* 50:182-192. 1910.
3. BABCOCK, S. M., Metabolic water; its production and rôle in vital phenomena. *Wis. Agric. Exp. Sta. Res. Bull.* 22. 1912.
4. BAILEY, C. H., and GURJAR, A. M., Respiration of stored wheat. *Jour. Agric. Res.* 12:685-713. 1918.
5. ———, Respiration of cereal plants and grains. II. Respiration of sprouted wheat. *Jour. Biol. Chem.* 44:5-7. 1920.

6. BLACKMAN, F. F., Optima and limiting factors. *Ann. Botany* 19:281-295. 1905.
7. CHACE, E. M., and DENNY, F. E., Use of ethylene in the coloring of citrus fruits. *Jour. Ind. Eng. Chem.* 16:339-340. 1924.
8. CHATIN, A., Études sur la respiration des fruits. *Compt. Rend. Acad. Sci. Paris* 58:576-579. 1864.
9. CLAUSEN, H., Beiträge zu Kenntniss der Athmung der Gewächse und des pflanzlichen Stoffwechsels. *Landw. Jahrb.* 19:893-930. 1890.
10. CROZIER, J. W., On biological oxidations as function of temperature. *Jour. Gen. Physiol.* 7:189-216. 1924.
11. DRAIN, B. D., Temperature and respiratory enzymes of apples. *BOT. GAZ.* 82:183-194. 1926.
12. DUVEL, J. W. T., The vitality and germination of seeds. *U.S. Dept. Agric. Bur. Pl. Ind. Bull.* 58. pp. 96. 1904.
13. GERBER, C., Recherches sur la maturation des fruits charnus. *Ann. Sci. Nat. Bot. Ser. 8.* 4:1-280. 1896.
14. GODLEWSKI, E., Über anaerobe Eiweisszersetzung und intramoleculare Atmung in den Pflanzen. *Bull. Acad. Sci. Cracovie* 8:623-704. 1896.
15. GORE, H. C., Studies on fruit respiration. *U.S. Dept. Agric. Bur. Chem. Bull.* 142. 1911.
16. HARRINGTON, G. T., Respiration of apple seeds. *Jour. Agric. Res.* 23:117-130. 1923.
17. HARVEY, R. B., Blanching celery. *Univ. Minn. Agric. Exp. Sta. Bull.* 222. pp. 20. 1925.
18. HILL, G. R. JR., Respiration of fruits and growing plant tissues in certain gases with reference to ventilation and fruit storage. *Cornell Agric. Exp. Sta. Bull.* 330. 1913.
19. KRAJNIK, B., Über eine Modifikation des Mikrorespirationsapparates. *Biochem. Zeitschr.* 130:286-293. 1922.
20. LEITCH, I., Some experiments on the influence of temperature on the rate of growth in *Pisum sativum*. *Ann. Botany* 30:25-46. 1916.
21. LOREE, R. E., The nutrient requirements of the strawberry. *Mich. Agric. Exp. Sta. Tech. Bull.* 70. 1925.
22. LUTHRA, J. C., Some experiments on the effects of dry and moist air on the rate of respiration and breakdown of ripe pears. *New Phytol.* 23:131-142. 1924.
23. MACK, W. B., The action of ethylene in accelerating the blanching of celery. *Plant Physiol.* 2:103. 1927.
24. MAGNESS, J. R., Physiological studies on apples in storage. Thesis. Univ. of Chicago. 1923.
25. MORSE, F. W., The effect of temperature on the respiration of apples. *Jour. Amer. Chem. Soc.* 30:876-881. 1908.

26. PALLADIN, W., Über den verschiedenen Ursprung der während der Atmung der Pflanzen ausgeschiedenen Kohlensäure. Ber. Deutsch. Bot. Ges. 23: 240-247. 1905.
27. REGEIMBAL, L. O., VACHA, G. A., and HARVEY, R. B., The effect of ethylene on the respiration of bananas during ripening. Plant Physiol. 2:357-359. 1927.
28. RHINE, LOUISA E., Divergence of catalase and respiration in germination. BOT. GAZ. 78:46-67. 1924.
29. KOLKWITZ, R., Über die Athmung ruhender Samen. Ber. Deutsch. Bot. Ges. 19:285-287. 1901.
30. SHERMAN, HOPE, Respiration of dormant seeds. BOT. GAZ. 72:1-30. 1921.
31. STEHLE, R. L., and McCARTY, A. C., Further data concerning the alleged relation of catalase to animal oxidations. Jour. Biol. Chem. 42:269-272. 1927.
32. DE VILLIERS, F. J., Physiological studies of the grape. Union So. Africa Dept. Agric. Sci. Bull. 45. 1926.
33. WILLAMAN, J. J., and BEAUMONT, J. H., The effect of accumulated carbon dioxide on plant respiration. Plant Physiol. 3:45-59. 1928.

# SPECIALIZATION IN SECONDARY XYLEM OF DICOTYLEDONS

## I. ORIGIN OF VESSEL

FREDERICK H. FROST

(WITH TWENTY FIGURES)

### Introduction

So relatively few existing angiosperms have been studied by morphologists and anatomists that adequate data are not available, at the present time, for constructing a comprehensive systematic classification of woods. It is true that more or less serviceable keys for the identification of commercial woods of certain specific regions have been formulated, but such keys cannot be relied upon in identifying woods of other regions, nor in identifying specimens from non-commercial species. Although the ultimate solution of the problem of classifying and identifying woods is dependent upon the compilation of larger volumes of reliable descriptive data, the investigation of the major trends of evolutionary specialization in the secondary xylem should be of considerable significance in arranging and interpreting these data and in visualizing the salient features of a natural classification. For example, if the evolutionary specializations of the flower and of the stem are closely correlated, the classification of the woods may well be made to parallel that based upon floral structures. On the contrary, if there is relatively little correlation in the evolutionary specialization of these organs,<sup>1</sup> an independent system of classification may have to be formulated for woods. A knowledge of the trends of specialization is also an aid in distinguishing between environmental variations and hereditary sequences in the individual, and makes it possible to refer specific hereditary variations to a definite scale, defined by the wide variation of evolutionary development.

<sup>1</sup> There is much evidence, both observational and theoretical, to indicate that such is the case. While the agreement between wood and floral structure in a genus is close in many cases, the degree of correlation becomes lower as the size of the classification unit is increased.

The writer plans to trace the major lines of specialization in the secondary xylem and to publish his results in a series of related papers. The present paper, the first of this series, discusses the origin of the vessel and the vessel segment; the second paper will deal with the specialization of the end wall of the vessel segment; the third with the changes which the lateral pitting of vessel segments undergo during evolutionary development; and the fourth with the form and distribution of vessel segments, etc. A list of new diagnostic characters and a discussion of their use in identification will be given in the final paper of the series.

### Methods

Four methods have been employed in studying the evolutionary development of the characteristics of wood. Although these methods are not new there has been so much confusion in their application that it seems desirable to restate them in detail.

The first method is the method of association, and may be described as follows. If it is possible to determine which of two characters is primitive, and if it be assumed that the two structures are genetically related in a direct line, then it follows that the primitive condition of the advanced structure will be similar to the general condition of the primitive structure. If it is found in the application of this method that such similarity does not exist to a considerable extent, it follows that the assumption of direct genetic relationship is incorrect, or that the two structures are so widely separated in the evolutionary scale that the primitive condition of the advanced structure is lost. If the similarity is close, however, both in the homologous and analogous sense, it follows that the assumption is correct, and it becomes easy to determine statistically the primitive condition of the advanced structure. This method is of use only when the characteristic in question is variable and when it is possible to obtain statistically sound samples from comparable regions.

The second method is as definite as the first and involves no complex assumptions. This is the method of correlation, and it assumes that in a given homogeneous tissue, such as the secondary xylem, there will be definite correlations, in the strict statistical sense, between the degrees of specialization of the chief characteris-

tics in a large random sample. That is, the rates of evolutionary development will be correlated; hence it follows that those characteristics which correlate with a primitive character, established as primitive by the method of association, will themselves be primitive, and those that correlate with an established specialized character will be specialized. This does not mean, of course, that a specialized and a primitive character cannot be found in the same tissue of a given species, since correlations are expressions of major trends or average relationships.

A correlation, unless it is perfect, is a description of an average or major trend. This implies that there are exceptions to the general rule if the population is studied from the individual rather than the group standpoint. This consideration leads directly to the third method, the method of exceptions. It is the study of the individual exceptions from the average line of specialization, when dealing with the development of specific characters, which will throw light upon special or unusual variations from the main course. The exceptions do not, of course, exist until the rule is established, and for this reason the method of exceptions is a corollary of the method of correlation. Both methods give evidence which may be directly interpreted: the latter the major lines of evolutionary development and the former the minor lines.

The statistical method is essentially a method for the study of the characteristics of a large group by taking individual samples, at random, from the group. Experience, in agreement with theory, has repeatedly shown that the accuracy of conclusions drawn from statistical results is proportional to the number of cases used in deriving the statistical constants. For this reason the number of individual woods measured and studied in these investigations is large in order to insure a fair representation of dicotyledonous woods in general.<sup>2</sup> Where the numbers were small, due to lack of material,

<sup>2</sup> The writer is fortunate in having access to the large collection of microscopic slides of woody plants which has been brought together and prepared under the direction of Professor I. W. BAILEY. This collection is representative of the woods of the world, and includes 63 of the 76 orders and 137 of the 264 families listed by HUTCHINSON. There are well over 800 genera and 2000 species in the collection. The writer has also drawn material from his own collection of the woods of the United States which at present includes some 150 genera and 450 species.

the results were safeguarded by statistical checks. The writer feels that investigators in this field have too often drawn rather sweeping conclusions from insufficient material.

THOMPSON (19) gives a detailed discussion of correlated specialization, his method of Cartesian co-ordinates in particular giving a graphic proof of the validity of the concept.

The fourth method of studying evolutionary trends is the method of sequences, and it is essentially concerned with the reconstruction of evolutionary variability from the observable variation of living forms. Variation in living forms may be ontogenetic, positional, irregularly disposed in a tissue, or may be found among the individual units of a taxonomic group. There is no fundamental distinction between the various types since all types are caused by differential rates of specialization and the persistence of primitive features.

GEGENBAUR (11), MORGAN (17), SCOTT (18), and others have shown that any particular sequence, regardless of type, may be cenogenetic, and that the direction of the specialization cannot be determined safely from a consideration of the sequence alone. For these reasons the attack on a given problem resolves itself into first determining by the methods of association and correlation which characters are primitive and which are specialized, and then constructing the intervening stages by an application of the method of sequences. When used in this manner the method is not subject to the criticisms which have been made against the doctrines of recapitulation, reversion, and conservative regions, since it is possible to distinguish between cenogenetic and palingenetic variations, and since the direction of the specialization is determined by independent methods.

The theories in regard to the origin of the vessel in the dicotyledons are various, and the literature is complicated, confused, and contradictory. No critical historical account is given at this stage of the presentation of the problem; on the contrary; it seems more logical to reattack it from the beginning by an application of the methods outlined, and then, with the knowledge which a broad survey of the secondary xylem of the dicotyledons gives, to enter into a detailed historical discussion.



### Initial considerations

The term vessel was concisely defined by VON MOHL (16) as follows:

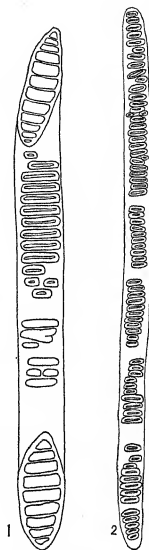
If this remains closed (the end wall) after its development is completed it is called a cell, *cellula*, but if a row of utricles arranged in a line becomes combined, during development, into a tube with an uninterrupted cavity, through the absorption of the cross walls, a compound elementary organ is produced, the *vessel*.

While this definition was accepted and widely used by many leading investigators of the nineteenth century, more recently the term vessel has been frequently misapplied. A vessel is clearly by, VON MOHL's definition, a series of individual cells, properly called vessel elements or vessel segments, which are joined end to end with perforate division walls.

A vessel segment (fig. 1) is chiefly distinguished from a tracheid (fig. 2) by the presence of an end wall, specifically distinct from the other walls, and by the presence of a perforation. The first point has been little emphasized in discussions concerning the origin of the vessel segment; its importance will be made clear in the discussion which follows. There is little fundamental difference in regard to the pitting of the vessel and the tracheid; both may have the typical elongate scalariform type of pit, the transitional type, or the opposite or alternate arrangement of the very common circular type.

### Discussion

It is of primary importance, in determining the manner in which the vessel segment originated, to establish which of the various types



FIGS. 1, 2.—Fig. 1, diagram of typical vessel segment with scalariform perforations and scalariform lateral pitting; fig. 2, *Trochodendron aralioides*, typical scalariform tracheid from secondary xylem.

of vessel segments is primitive. This can be accomplished by an application of the methods of association and correlations if we grant that the following assumptions are essentially correct: (1) that the theory of evolution is correct; (2) that specialization results in a series of graded forms; (3) that the tracheid is a more primitive structure than the vessel segment; and (4) that the vessel segment is genetically related to and derived from the tracheid. The correctness of the first and second assumptions is almost beyond doubt; the third is supported directly by evidence from the fossil record and by considerable collateral evidence; the fourth is universally held to be true since tracheids and vessel segments serve the same function, are in the same positional arrangement, and are morphologically similar. We need not accept these assumptions as facts, however, since if they are incorrect there will be a decided lack of coherence in the data. If, however, it can be shown that the many facts which it is possible to assemble become unified under these assumptions it further establishes their correctness.

If these assumptions are considered to be true, it follows, by an application of the method of association, that the truly primitive vessel segment will be characterized by the features which define tracheids in general. It must be remembered in this connection, however, that secondary vessel segments must be compared with secondary tracheids; that comparable regions of the plants must be used for comparison. What, then, are the characteristics which tracheids, as a whole, possess which should be incorporated in the organization of the primitive vessel segment? In brief there are six features: (1) great length, (2) small cross-sectional area, (3) angularity of outline, (4) thin walls, (5) constant width of walls in transverse section, and (6) the absence or very slight development of an end wall.

1. GREAT LENGTH.—Tracheids are much longer than vessel segments. Table I gives the averages computed from measurements given by BAILEY and TUPPER (3). These figures represent, as a rule, lengths of vessel segments and tracheids taken from mature secondary wood, and the grand averages are computed from averages of each species.

It follows, in view of the great differences in length of tracheids

and vessel segments, that primitive vessel segments will be long, and by the method of correlation, that the characteristics of long vessel segments will be primitive. BAILEY and TUPPER (3 table II) indicate the type of perforations possessed by long (primitive) vessel segments.

In some 270 odd species from 114 families there are seventeen species listed whose vessel segments average more than 1.2 mm., and all have exclusively scalariform perforations. In other words, the correlation between very long vessel segments and scalariform end walls is positive and perfect, or, in statistical terms, the correlation

TABLE I

| GROUP              | NO. OF SPECIES | TRACHEID<br>LENGTH (MM.) | VESSEL SEGMENT<br>LENGTH (MM.) |
|--------------------|----------------|--------------------------|--------------------------------|
| Cordaitales.....   | 2              | 5.05                     | .....                          |
| Bennettitales..... | 2              | 5.25                     | .....                          |
| Cycadales.....     | 1              | 6.80                     | .....                          |
| Ginkgoales.....    | 1              | 3.50                     | .....                          |
| Coniferales.....   | 146            | 3.64                     | .....                          |
| Gnetales.....      | 3              | .....                    | 0.96                           |
| Dicotyledons.....  | 276            | .....                    | 0.57                           |

coefficient is  $-1$ . Again, of the forty-six species whose vessel segments average 1 mm. or more, thirty-two have entirely scalariform perforations, six have scalariform-porous perforations, and eight have oblique porous perforations with vestiges of the scalariform condition. Not one of these species has vessel segments with the typical transverse porous end wall. For contrast, at the lower end of the scale there is a perfect correlation between transverse porous perforations and shortness. In the thirty-two species whose vessel segments average 0.02 mm. or less, all have transverse porous perforations. An analysis of the correlation in the 274 species is shown in table II.

In this particular case the evidence is overwhelmingly in favor of the scalariform type of perforation being the most primitive.

2. SMALL CROSS-SECTIONAL AREA.—Since on the average tracheids are much smaller in cross-sectional diameter than vessels, it follows, by the method of association, that the primitive vessels

will be small,<sup>3</sup> and by the method of correlation that the characteristics of small vessels will be primitive. Tracheids in general average about 0.03 mm. in diameter. Forty dicotyledonous species with scalariform perforations, picked at random from the collection, average 0.067 mm. in diameter, whereas forty diffuse porous woods, with transverse porous perforations, average 0.12 mm. In other words, the long vessel segments with exclusively scalariform end walls are also small in diameter similar to tracheids, while the short vessel segments with transverse porous perforations are large in diameter dissimilar to tracheids.

TABLE II  
ANALYSIS OF SPECIES

| PERFORATIONS                               | VESSEL SEGMENT LENGTH |             |             |             |             |
|--|-----------------------|-------------|-------------|-------------|-------------|
|  | 1.3-2.0 mm.           | 1.0-1.3 mm. | 0.6-1.0 mm. | 0.3-0.6 mm. | 0.0-0.3 mm. |
| Scalariform.....                           | 17*                   | 15          | 17          | 5           | 0           |
| Scalariform-porous.....                    | 0                     | 0           | 7           | 5           | 0           |
| Porous-oblique, vestigial scalariform..... | 0                     | 8           | 12          | 14          | 0           |
| Transverse-porous.....                     | 0                     | 0           | 30          | 106         | 32          |

\* In almost all cases only one species is taken from each genus. Since the species of a genus are usually alike as regards wood structure, it would give improper weight to certain types to increase the number of cases by taking more than one representative from each genus.

It will be noted that only diffuse porous woods were used in making these measurements, to afford a better comparison with the purely scalariform woods which are necessarily diffuse porous. Ring porous woods tend to have larger vessels than diffuse porous woods. There is a high correlation between the diffuse porous condition and the presence of scalariform perforations. As one might expect, the distribution of primitive vessels follows the distribution of tracheids (that is, is diffuse, or more or less evenly spaced throughout the xylem). These data indicate that the scalariform perforation is primitive, a result in complete harmony with the conclusions obtained from the length data.

3. ANGULARITY OF OUTLINE.—We should also expect that the primitive vessel segment would be angular in transverse outline since tracheids in general are angular. The forty scalariform per-

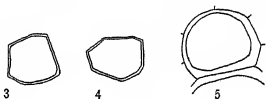
<sup>3</sup> BROWN (8) has recently reached this same conclusion.

forate species studied in regard to cross-sectional diameter must therefore be angular if the previous conclusions are correct. As a matter of fact all are angular in outline, or the correlation between the two primitive characteristics, angularity and small diameter, is perfect in these species. Of the forty porous perforate species, six are angular while thirty-four are round or oval. The correlation between great length and angularity is also perfect since the seventeen species with vessel segment lengths averaging over 1.2 mm. have angular vessels in all cases. Fig. 3 illustrates an angular tracheid from *Sequoia sempervirens*, and fig. 4 an angular vessel from *Gordonia lasianthus*. This is further proof that the scalariform perforation is a primitive feature in the wood of the dicotyledons.

4. THIN WALLS.—In nearly all cases tracheids possess evenly thickened walls, and if the forty supposedly primitive woods are truly primitive they must also have vessels with evenly thickened walls. That this is true is shown by the following figures. Of the forty woods with scalariform perforations, thirty-nine have evenly thickened walls, in contrast to the forty porous perforate species in which only nine have evenly thickened walls. Fig. 5 illustrates the unevenly thickened vessel type from *Exothea paniculata*, which may be compared with the evenly thickened type shown in fig. 4.

5. CONSTANT WALL WIDTH.—Tracheids are characterized by thin walls, and in this case all of the forty scalariform species are thin-walled. In the porous group ten have thin walls, thirteen have vessels which are thick-walled when in contact with other vessels, and seventeen have thick walls only.

6. UNDEVELOPED END WALL.—In the scalariform types the end wall is highly inclined or absent, and does not materially change the characteristic tracheid-like shape of these primitive vessel segments. In the porous species the end wall is usually transverse, and greatly



FIGS. 3-5.—Fig. 3, *Sequoia sempervirens*, angular tracheid in transverse section; fig. 4, *Gordonia lasianthus*, angular vessel in transverse section; fig. 5, *Exothea paniculata*, common type of vessel with unevenly thickened walls.

alters the shape of the vessel segment from the original tracheid-like form.

In summary, the vessel segments which retain the tracheid characteristics of length, small diameter, angularity of outline, evenly thickened walls, thin walls, and an unspecialized end wall are found, as they should be if all these characteristics are primitive, in the same group of plants, and are dominantly characterized by the presence of scalariform perforations similar to those found in *Pteris* and totally different from the Gnetalian type. The conclusion is irresistible that the scalariform perforation is the most primitive type found in the dicotyledons.

Now that the nature of the primitive perforation has been determined, its mode of origin from the tracheid may be discussed. Since there is a close agreement between the morphology of certain vessel segments and tracheids in general, indicating that this type of vessel segment must be primitive, it is logical to infer that there will be a marked agreement between the pitting of these vessel segments and the pitting of the tracheid type from which they were evolved by evolutionary specialization. It is not known, of course, which type of pitting characterized these tracheids, since the pitting of tracheids is variable, but we can discover what type dominates in the primitive vessel segments, and we may conclude that this type will be similar to that from which it was produced. This is a reverse application of the method of association.

Of the seventeen species previously referred to, with very long vessel segments, fourteen are characterized by scalariform lateral pitting and three by transitional to opposite pitting. This is indeed meager evidence, due to the small number of cases, but it is a decided indication of correlation. BAILEY and TUPPER have supplied abundant evidence, however, which is summarized in table III.

In group 1 the scalariform lateral pitting is dominant, in group 2 transitional and opposite pitting dominates, in group 3 opposite and alternate pitting dominates, and in group 4 alternate pitting is most common.

The writer's observations, based upon 104 scalariform, scalariform-porous, and vestigial scalariform woods, gives the results shown in table IV.

Tables III and IV show that there is a high correlation between the primitive scalariform condition of the end walls and the scalariform condition of the side walls. It is rather interesting to note

TABLE III  
ANGIOSPERMAE—DICOTYLEDONEAE  
(BASED ON 274 SPECIES IN 114 FAMILIES)

| TYPES OF LATERAL PITTING                   |                                       |            |
|--|---------------------------------------|------------|
| PERFORATIONS                               | LATERAL PITTING                       | PERCENTAGE |
| (1) Highly inclined, entirely scalariform  | Scalariform and opposite.....         | 86         |
|  | Opposite and alternate or alternate.. | 14         |
| (2) Inclined, scalariform, porous          | Scalariform and opposite.....         | 80         |
|  | Opposite and alternate or alternate.. | 20         |
| (3) Inclined porous, vestigial scalariform | Scalariform and opposite.....         | 11         |
|  | Opposite and alternate or alternate.. | 89         |
| (4) Transverse porous                      | Scalariform and opposite.....         | 6          |
|  | Opposite and alternate or alternate.. | 94         |

that in group 1 of the table given by BAILEY and TUPPER, 83 per cent of the species have other tracheary cells with distinct bordered pits, whereas in group 4 only 17 per cent of the species have distinct bordered pits in their fibrous elements. The scalariform condition of

TABLE IV  
TYPES OF LATERAL PITTING

| PERFORATIONS  | LATERAL PITTING  | PERCENTAGE |
|---|------------------|------------|
| (1) Highly inclined, entirely scalariform                   | Scalariform..... | 57         |
|   | Opposite.....    | 33         |
|   | Alternate.....   | 10         |
| (2, 3) Inclined scalariform-porous or vestigial scalariform | Scalariform..... | 19         |
|   | Opposite.....    | 27         |
|   | Alternate.....   | 54         |

The transitional type of pitting is classed as scalariform or opposite, depending upon which it most nearly resembles.

both the end and the side walls therefore correlates with the accepted primitive type of fiber pitting.

The only interpretation which can be given these data is that scalariform lateral pitting is primitive in the organization of the

vessel segments of the dicotyledons. There is, then, good reason to suppose that the tracheid type from which these primitive vessel segments were derived was characterized by scalariform lateral pitting. In other words, the vessel segment originated in the dicotyledons in precisely the same manner as it originated in *Pteris*, by the loss of membranes from the scalariform pits at each end of the tracheid and the formation of an end wall.

If this reasoning and interpretation are correct, one should expect to find, in the secondary xylem of primitive dicotyledons, transitions from scalariform tracheids to scalariform vessel segments, which would record the evolutionary change. An examination of a great number of woods failed to reveal any evidence that such transitions occur in the secondary xylem, or that true scalariform tracheids are present in the secondary wood of forms which possess vessels. This can mean either that the theory is entirely incorrect or that the secondary xylem is so highly specialized that such transitions have been lost. The writer's attention next centered on the primary xylem, and it was at once clear that, in species which possess a primitive secondary xylem, such transitions not only occur but are also in positional sequence from the pith outward, and show, in great detail, the transition from the scalariform tracheid to the scalariform vessel segment. If this is a true evolutionary sequence, there must be some reason why it occurs in the primary and not in the secondary wood, and why the transition is in positional sequence.

Measurements and examinations were accordingly made of the primary wood of a great number of species. The results are summarized in table V.

It will be noted that the dicotyledons readily segregate into three major groups, and that group 2 is composed of species with primitive scalariform perforations in the primary xylem, and specialized porous perforations in the secondary xylem. This means that in this group the secondary xylem is more highly specialized than the primary. In the first protoxylem group vessels are frequently absent, a fact which further substantiates this opinion.

In the protoxylem, metaxylem, and secondary xylem of the first annual ring, the vessel segments decrease in length in groups



1 to 3, as the end wall becomes more highly specialized. These correlations correspond exactly with the data, previously given, for the mature secondary xylem. There is, then, striking evidence to show that the evolution of the vessel proceeded along the same lines in these three regions.

In all three classes there is also a reduction in average length from the protoxylem, to the metaxylem, to the secondary xylem when each region is considered as a unit. Since length is a primitive characteristic, it may be concluded that the vessel segments of the protoxylem are less specialized than those of the metaxylem, and

TABLE V  
TRACHEID AND VESSEL SEGMENT LENGTHS\*

| PERFORATIONS   | PROTOXYLEM<br>(MM.) | METAXYLEM<br>(MM.) | FIRST FORMED<br>SECONDARY<br>XYLEM (MM.) |
|--|---------------------|--------------------|--|
| (1) Absent or scalariform in primary xylem,<br>scalariform in secondary xylem..... | 1.948               | 1.647              | 0.709                                    |
| (2) Scalariform in primary xylem, porous in<br>secondary xylem.....                | 1.331               | 1.203              | 0.426                                    |
| (3) Primary porous, secondary porous.....  | 0.974               | 0.751              | 0.338                                    |

\* These figures are average lengths of scalariform tracheids, when vessels are absent, or of vessel segments.

that those of the metaxylem are less specialized than the vessel segments of the secondary xylem. It will be noted that the greatest average lengths occur in the protoxylem and metaxylem of group 1, and it was in species of this class that the tracheid to vessel segment transitions were found.

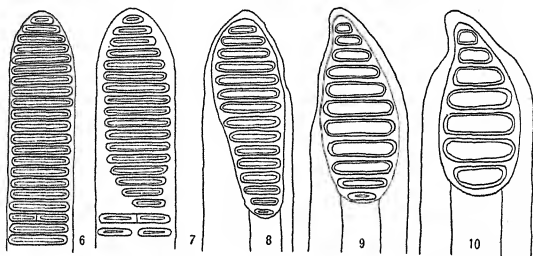
While the measurements in this instance apply to the protoxylem and the metaxylem as a unit, only the first formed secondary xylem was considered. In any given angiosperm, as a rule, there is a tendency for the cambial initial to increase in length with increasing age. This increase in length causes an increase in the length of the corresponding vessel segments, since, as BAILEY (1) has shown, there is a close correlation between vessel segment and cambial initial length. While the increase is not great, it tends to raise the length average of the secondary xylem as a whole, and to make the differences between the means of the secondary xylem and the metaxylem more comparable with the differences between the means of the

protoxylem and metaxylem. While there is no doubt that the vessels of the secondary xylem are more highly specialized than the vessels of the primary xylem, it would seem fair to argue that the vessels of the mature wood are less specialized than the vessels of the early secondary xylem, since the vessel segment length is longer in the former region. While this argument superficially appears to be sound, a consideration of the ontogenetic variations in primitive plants in general shows it to be false. For example: vessel segment length averages about 1 mm. in the mature wood of scalariform perforate species, and this is more tracheid-like than the young wood with an average vessel segment length of only 0.7 mm., but when this mature wood average is compared with the average tracheid length of the mature wood of the gymnosperms, it is no more tracheid-like than the young wood average, since the tracheids of the gymnosperms also increase in length with increasing age of the tree. Relatively the comparison is closer between the young regions, since the tracheids of the gymnosperms increase to a greater extent with age than the vessel segments of the angiosperms. The increase in length which the cambial initial undergoes during its life history is, evidently, a general phenomenon which is correlated with the arboreal habit and which should be rendered constant when comparing average lengths. This can be accomplished by treating the secondary xylem as a unit, or by comparing young wood with young wood and old wood with old wood.

The data presented in table V indicate that the reason no transitions from the scalariform tracheid to the scalariform vessel segment were found in the secondary xylem is that the vessel segments in this tissue are so highly specialized that such transitions have been lost. The reason the transition is still present in the primary wood is explained by the very strong probability that the primary wood is much less specialized, in respect to the type of vessel segment, than the secondary wood, a supposition which agrees with all the facts.

Before proceeding with a discussion of the details of this transition, it is possible further to verify this hypothesis. If the interpretation of the data of table V is correct, this protoxylem to secondary xylem transition should reflect the accepted sequence from

scalariform to porous perforations; that is, if the reasoning holds for one case it should hold for another. As a matter of fact, an inspection of almost any plant with scalariform perforations in the primary wood and porous perforations in the secondary wood will show this transition in considerable detail. The details of this line of specialization will be covered in the next paper of this series; it is only necessary at this time to show that there is complete harmony in the evidence. This sequence of tracheid to vessel segment transition is shown in the following genera: *Ilex*, *Nyssa*, *Viburnum*, *Cornus*, *Rhododendron*, *Schizandra*, *Gordonia*, *Symphlocos*, and many others.



FIGS. 6-10.—*Schizandra chinensis*, transitional stages showing origin of vessel from scalariform tracheid.

In *Schizandra chinensis* the transition is especially striking, since the vessel segments of the secondary wood have a rather highly specialized type of scalariform perforation, quite distinct from the primitive type found in the metaxylem and late protoxylem. The spiral protoxylem elements are tracheids since they do not possess an end wall or a perforation. The region between the protoxylem and metaxylem is characterized by long tracheids whose lateral walls are marked by distinct scalariform pits. A tracheid of this type is illustrated in fig. 6, and it will be seen that no end wall has developed. There is no difference in the pitting of these pre-vessel types of tracheids and the scalariform lateral pitting of vessel segments. The pitting at the middle is the same as the pitting at the

ends, and there is no indication of the loss of pit membranes. Fig. 7 represents the next type of cell in this positional and evolutionary sequence. That this cell is neither a typical tracheid nor a typical vessel segment is evident. No end wall has as yet developed, but the scalariform pitting at the ends of the cell has taken the form of a perforation, while the lateral pitting is breaking up into the transitional condition. These cells are long, their diameter is small, their walls are thin and evenly thickened, their outline angular, and the end wall is unspecialized, characteristics which are common to both tracheids and primitive vessel segments.

Fig. 8, taken from the middle of the metaxylem, illustrates the next step in this series. Here the perforation has been formed by the loss of the pit membranes from the scalariform pits at the end of the cell resulting in a vessel segment, which still, in general, retains tracheid-like characteristics. Fig. 9, from the end of the metaxylem, shows the further changes which occur at the end of the cell: the increase in diameter, the establishment of the end wall, the reduction in the number of bars of the perforation, and the widening of the apertures resulting in a vessel segment, much more vessel-like than tracheid-like. These cells are, of course, much shorter than those illustrated in figs. 6 and 7. Fig. 10 represents the end product of the series, the typical vessel segment of the secondary wood of *Schizandra chinensis*. The original scalariform lateral pitting has been replaced at this stage by the transitional to opposite pitting characteristic of the secondary wood. Vestigial perforations, as one would expect, are found occasionally in the secondary wood. These are similar to those illustrated in fig. 8, but the scalariform tracheids have entirely disappeared.

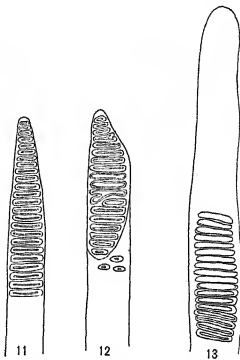
Figs. 11 and 12 show the origin of the vessel as reflected in *Nyssa aquatica*. The contrast between the scalariform tracheid and the end product is not so striking in this case because the vessel segments of the secondary xylem are not so highly specialized as they are in *Schizandra*.

In view of the proof offered that the transition from the protoxylem to the secondary xylem represents a primitive to specialized sequence which is palingenetic, and the accurate portrayal this sequence gives in the change from scalariform to porous perforation,

there is little reason to doubt that the detailed transitions here illustrated give a connected picture of the evolution of the scalariform vessel segment from the scalariform tracheid.

In primitive woods which show the tracheid to vessel segment transition, the elements which are intermediate between tracheids and vessels generally show no sharp transition from the scalariform condition of the end wall to the scalariform condition of the side wall. It follows that this arrangement should occasionally be found in the secondary vessel segments of species with primitive wood structure, and the point is clearly illustrated in fig. 13 from *Laurelia novae-zelandiae*, where this condition is somewhat common in the secondary wood. This is a very primitive arrangement, of course, and therefore there should be a correlation between this characteristic and great length. To test this, the species with exclusively scalariform perforations were divided into two groups: (1) those with a gradual transition in the type of pitting from the end to the lateral wall of the vessel segment; and (2) those with a sharp transition in the type of pitting. The lengths of the vessel segments in these two groups were then measured and the averages are given in table VI.

In the first group the shortest vessel segment is 0.7 mm., and twenty-two species have vessel segments which average 1 mm. or more. In the second group the shortest average vessel segment length is 0.4 mm., and only eight species average 1 mm. or more (length data taken from table II of BAILEY and TUPPER). It will be seen that this primitive characteristic, which is really a combina-



FIGS. 11-13.—Figs. 11, 12, *Nyssa aquatica*, transition showing development of scalariform perforation from scalariform tracheid; fig. 13, *Laurelia novae-zelandiae*, true palingenetic sequence from scalariform lateral pitting to scalariform openings.

tion of two primitive characters, is closely correlated, as one would expect, with great length.

An interesting point in this connection was made by BROWN (7), who argued that the rate of specialization of the end wall would be slower than the rate of change of the pitting of the side walls, because the end walls would be in contact with vessel segments only, whereas the side walls would be in contact with a variety of cell types. While BROWN's point is well made, as evidence to be presented in a later paper will show, it must be remembered that there

TABLE VI

| TYPE   | NO. OF SPECIES | AVERAGE LENGTH<br>(MM.)* |
|--------|----------------|--------------------------|
| 1..... | 29             | 1.27                     |
| 2..... | 22             | 0.87                     |

\* Average length of vessel segments with scalariform perforations is 1.09 mm. Average length of 1.27 mm. of vessel segments with both scalariform side and end walls really represents a multiple correlation, since it is the mean of the prediction of length from two factors. That it is greater than the prediction of length from scalariform ends alone is to be expected both statistically and logically.

are a number of species with primitive scalariform pitting on both end and side walls, and that the difference in rate of change, while distinct, is not of great magnitude and does not have much effect on the correlation between side and end wall characteristics.

So far no mention has been made of the magnoliaceous genera *Trochodendron*, *Tetracentron*, and *Drimys*, in which vessels are absent in the wood. No discussion of the origin of the vessel would be complete without a consideration of these very interesting and unusual forms.

*Trochodendron* is found in Japan and Formosa and *Tetracentron* in central and western China. The individuals of these monotypic genera reach a considerable size and are found in moist montane habitats. *Drimys* is rather widely distributed in the southern hemisphere and is found as a small tree or shrub growing under somewhat mesophytic conditions.

All three genera have a primary and secondary xylem which is composed entirely of tracheids and parenchyma. There is no indication of the formation of perforations or end walls. The wood of these

genera is unquestionably very primitive, and represents an offshoot from that group of plants which gave rise to the angiospermous type of wood by the acquisition of vessels. The type of tracheid which they possess should therefore be quite similar to the type from which the dicotyledonous vessel segment was derived. A study of the histology of their tracheids should therefore give an independent check on this theory of the origin of the vessel.

*Trochodendron* has mature secondary tracheids which average 4.5 mm. in length; *Drimys* has secondary tracheids which average 4.3 mm. in length; and in *Tetracentron* the average length is 1.8 mm. in the first annual ring, which indicates that the mature wood average is well over 3 mm. These averages are more than twice as long as any observed in primitive dicotyledons with vessels, and these facts substantiate the opinion that great length is a primitive characteristic, and that the vessel segment was derived from a long tracheid.

Since it has been found that scalariform lateral pitting is primitive, and that the type of tracheid which gave rise to the vessel segment was characterized by scalariform pitting, it would seem to follow that the tracheids of these genera should have scalariform lateral pits. The tracheids of the primary wood, with the exception of the spiral protoxylem elements, are scalariform and longer than the first formed secondary tracheids. The tracheids of the secondary xylem have either scalariform or circular pits; in *Tetracentron* and *Trochodendron* the former condition is common in the spring wood and the latter in the summer wood (figs. 2, 14); in *Drimys* the circular type of pitting predominates in the older secondary xylem. In these genera, as in the primitive dicotyledons with vessels, the primary wood specializes more slowly than the secondary xylem, and it is therefore quite apparent that the scalariform type of lateral pitting is more primitive than the circular type. The only difference between the scalariform tracheids of the primary wood of these species and the scalariform tracheids found in the primary wood of the primitive dicotyledons with vessels is that of length.

In conclusion, while these genera may well represent a blind line of specialization, there is every indication that they still retain many of the characteristics of the plants which gave rise to the dicotyle-

dons as we now know them, and that the scalariform tracheids of their primary wood are very similar to the type which gave rise to the vessel segment and thereby the vessel. The writer feels secure in venturing the prediction that angiospermous fossil wood will eventually be found which will show, in the secondary xylem, all the stages in the formation of vessel segments from long scalariform tracheids.

In the preceding pages an attempt has been made to bring together all the available evidence bearing on the origin of the vessel,

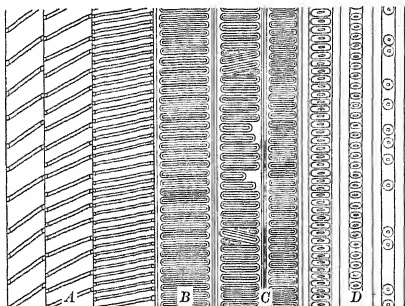


FIG. 14.—*Trochodendron aralioides*: (a) spiral protoxylem elements; (b) scalariform tracheid from metaxylem; (c) scalariform tracheids from spring wood of secondary xylem; (d) circular pitted tracheids from summer wood of secondary xylem. Spring wood tracheids of each annual ring are scalariform, while those found in summer wood are marked with circular pits.

and to evaluate this evidence in accordance with the principles outlined in the introduction.

It has been shown that the six features which are common to tracheids in general are all incorporated in the organization of vessel segments with scalariform perforations, and that these vessels also have scalariform lateral pitting. These facts led to the conclusion that the scalariform condition of the end and side wall is a primitive characteristic, and that the tracheid type which gave rise to the vessel segment had scalariform lateral pitting. It was then found

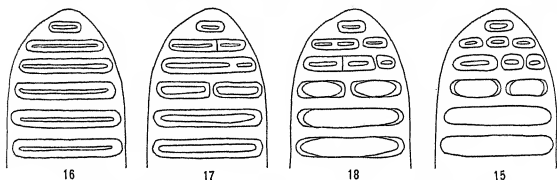


that certain genera, which are primitive in these respects, possessed sequences which showed in detail the stages in the evolution of the vessel segment from the scalariform tracheid. The histology of the tracheids of the primitive vessel-less genera of the Magnoliales indicates independently that these conclusions are correct. These facts are the main points in the argument that the scalariform perforation developed from scalariform lateral pitting by the loss of pit membranes from the ends of the tracheid, and form, with many minor facts, a unified whole when considered in the light of this theory. Why then, if the story of the origin of the vessel has been so clearly written in the secondary and primary xylem of existing dicotyledons, has there been such diversity of opinion in regard to this fundamental point? The writer will endeavor to illustrate in the following historical discussion that the lack of agreement is not due to the existence of contradictory facts, but is a result of the general use of methods which do not give reliable results when used independently of the methods of association and correlation.

#### Historical discussion

Although DE BARY (9), as early as 1884, suggested that the scalariform perforation is a modification of the end of a scalariform tracheid by the loss of the pit membranes, the first detailed theory of the origin of the vessel was described by BOODLE and WORSDELL (6) for the Gnetales and certain representatives of the dicotyledons. They considered, in the case of the dicotyledons, that the perforation developed by the fusion of circular pits into scalariform pits, which in turn lost their membranes and became scalariform openings. The theory was based upon transitions from circular pits to scalariform pits to scalariform openings, which they found and illustrated in several distinct cases. While such transitions occur, it is difficult to find any justification for interpreting the direction of the specialization as from circular pits to scalariform openings, rather than from scalariform pits to circular pits, or for assuming that the sequence is truly palingenetic rather than cenogenetic. Fig. 15 illustrates a sequence of this nature which in the light of present knowledge is rather easy to interpret. While it is known that the scalariform opening is derived directly from the scalariform pit, the data

in tables III and IV show that the scalariform and not the circular pit is primitive. Table VI also shows that the vessel segments with both scalariform lateral pitting and scalariform perforations are decidedly more primitive than vessel segments with circular lateral pitting and scalariform perforations. Figs. 16-18 illustrate diagrammatically the course of events which lead to the condition shown in fig. 15. Fig. 16 shows the now familiar scalariform tracheid which is to give rise to the vessel segment. In this case the three upper scalariform pits do not lose their membranes during the development and specialization of the end wall, but specialize as



FIGS. 15-18.—Diagrammatic illustration of formation of cenogenetic sequence

pits. This specialization results in a change from the primitive scalariform arrangement to the advanced opposite circular one. Figs. 17 and 18 show how this process occurs. The lower three scalariform pits of fig. 15 lose their membranes and specialize as scalariform openings; a specialization which results, as will be shown in detail in the next paper of this series, in the complete loss of the borders around the openings but with only a slight change in their scalariform shape. The sequence shown in fig. 15 must therefore frequently occur as a result of these two lines of specialization occurring at the same time with slightly different rates. The transition is accordingly of no particular evolutionary consequence. In the more primitive vessel segments, before sufficient time has elapsed for the primitive scalariform pits to break down into the opposite condition, the transition is truly palingenetic (fig. 13). The course of events is quite naturally the same, if one considers transitions from the lateral to the end wall. The interpretation of BOODLE and

WORSDELL is therefore incorrect, and affords an illustration of the dangers encountered when it is considered a sound procedure to interpret a sequence without first determining whether it is truly palingenetic and which end of the sequence is primitive.

In 1917 JEFFREY (13) accepted the theory advanced by BOODLE and WORSDELL. He based his conclusions entirely upon positional sequences of the type shown in fig. 15, and argued that opposite pitting is more primitive than scalariform because the sequence shows that opposite pits fuse to form scalariform pits. He figured and described the truly palingenetic sequence (fig. 13) in *Liriodendron*, and argued that this transition is not primitive since the former sequence proves that opposite pitting is primitive. If one used the same line of argument and started with the *Liriodendron* transition the conclusions would be exactly reversed, and by chance correct.

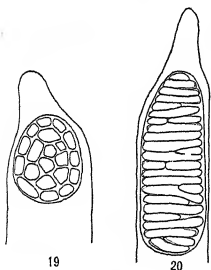
In 1918 THOMPSON (20), after an intensive study of the Gnetales, confirmed the opinion of BOODLE and WORSDELL regarding the evolution of the vessel in this group, but suggested that the angiospermous vessel might have developed from a scalariform type of tracheid by the loss of the pit membranes. Since THOMPSON was able to show, without deciding that this theory was correct, that the further specialization of the vessel segment in the angiosperms was quite different from that in the Gnetales, he allowed the matter to rest.

In the same year BROWN (7) published evidence to show that the primitive vessel segment, as is the case in *Pteris*, is merely a modified scalariform tracheid, similar to those found in *Tetracentron*. BROWN's paper is little more than a preliminary report, without illustrations, and it is a little difficult to understand why he did not present his evidence in detail in a later paper. He found transitions, in the dicotyledons, from the scalariform tracheid to the scalariform vessel segment, which he interpreted as a reflection of the phylogenetic origin of vessels. He also argued that scalariform lateral pitting is primitive since it is characteristic of conservative regions, since it is more characteristic of primitive families, and since transitions from scalariform to multiseriate lateral pitting are common in the angiosperms from the pith outward. The writer feels that the first two of the last three arguments have no foundation in fact, and that

the last argument is worthless unless it can be shown, on independent grounds, that scalariform lateral pitting is primitive. While the conclusions which BROWN reaches are correct, one cannot feel that they are based upon sound evidence.

In 1918 BAILEY and TUPPER (3) presented carefully compiled statistical data showing the true relationships between the various types of pitting and clearing up many important points. Unfortunately the significance of their results was overlooked by succeeding investigators, otherwise the whole question would have been settled several years ago.

Miss BLISS (5) upheld the theory advanced by BOODLE and WORSDELL and JEFFREY, and presented the same arguments. She also suggested a new type of origin for the vessel in which the vessel originated by the haphazard fusion of circular pits. This process is supposed to have given rise to vessel segments with the curious netlike perforations (fig. 19) similar to those of *Gnetum*. If this theory were correct this type of perforation would be characteristic of scalariform woods, which she admits are more primitive than scalariform-porous woods. As a



FIGS. 19, 20.—Fig. 19, *Brysonima lucida*, gnetum-like type of perforation; fig. 20, *Andromeda ferruginea*, perforation with branching bars.

matter of fact she draws her illustrations largely from scalariform-porous woods, and a study of a great number of scalariform and scalariform-porous woods shows that this type of perforation is found almost exclusively in the latter group. In the seventeen species previously cited, with very long vessel segments, the *gnetum*-like perforations do not occur.

Miss BLISS also considers that the scalariform perforation with branched bars is a form of the netlike perforation, caused by the haphazard fusion of certain circular pits and the regular fusion of other circular pits (fig. 20). The branching of bars is characteristic of all types of scalariform perforations, and is, as BROWN (7) sug-

gests, merely a result of the formation of scalariform perforations from irregular scalariform pitting.

In 1921 Miss MACDUFFIE (15) gave further illustrations in favor of the theory advanced by Miss BLISS. She found that the netlike perforations were characteristic of the Compositae, among other highly specialized groups, and on this basis one would be forced to conclude that the Compositae possessed a very primitive type of stem or grant that the netlike type of perforation was highly specialized.

In 1923 THOMPSON (21) again attacked this problem and confirmed the views of BROWN. Although he overlooked the significance of the study by BAILEY and TUPPER, THOMPSON based his conclusions upon a study of a number of species, and found, as the writer has found, that there is no evidence that the netlike type of perforation is primitive. It is evidently an anomalous oddity resulting from the breaking down of vestigial scalariform perforations in scalariform-porous woods.

That the matter was still unsettled in 1925 is evident from the account of the origin of the vessel given by EAMES and MACDANIELS (10), which can be interpreted in favor of either theory.

In the literature concerning *Trochodendron*, *Tetracentron*, and *Drimys* there is considerable diversity of opinion. GROOM (12) suggested that these genera were degenerate and had at one time possessed true vessels. THOMPSON and BAILEY (22) came to the conclusion that these genera represent a very primitive condition and have never possessed vessels. JEFFREY and COLE (14) questioned the conclusions of THOMPSON and BAILEY, and reached a position similar to that suggested by GROOM. In 1918 BAILEY and THOMPSON (4) reviewed the entire question and confirmed their earlier results, and later BAILEY and TUPPER (3) gave the average lengths of the tracheids of these forms.

JEFFREY and COLE found in an injured root of *Drimys* an element, without perforations, but with scalariform lateral pitting which they considered an indication of the previous possession of vessels in these genera. At the time they were not informed of the normal occurrence of scalariform tracheids in the secondary xylem of this group. It is difficult to understand why they should

reason that scalariform lateral pitting is typical of a vessel and not of a tracheid, since they must have been aware of the very wide occurrence of scalariform tracheids in both fossil and recent plants. It would seem rather, if one is to use the principle of reversion, that the only justifiable conclusion is that the ancestors of these extraordinary types possessed a secondary xylem composed of scalariform tracheids. Since their whole argument depends upon this doubtful interpretation and the erroneous conception that opposite pitting is more primitive than scalariform pitting, their results cannot be taken as justifying the conclusion that these forms once possessed vessels.

Highly specialized woods occur in the Cactaceae and Crassulaceae in which vessels are partially or almost completely suppressed, due to adaptation to extreme environmental situations. In cases of this nature the vessel segments are replaced by peculiar structures which are short and quite obviously specialized, and which retain evidences of their origin from vessel segments. There is no similarity between woods of this type and the secondary xylem of *Trochodendron*, *Tetracentron*, and *Drimys*. It would be a remarkable reduction which resulted in regular and very long scalariform tracheids from short vessel segments in large arborescent plants. BAILEY and THOMPSON give a complete discussion of the evidence presented by JEFFREY and COLE in favor of the interpretation that these genera are truly primitive.

### Summary

1. Tracheids are characterized by great average length, small cross-sectional diameter, angularity of outline, evenly thickened walls, thin walls, and the absence of a distinct end wall.
2. Vessel segments which retain these primitive characteristics nearly always have scalariform perforations; the scalariform perforation is therefore primitive.
3. There is a high correlation between the diffuse arrangement of vessels and the scalariform condition of the end wall.
4. Vessel segments with scalariform perforations are characterized by scalariform lateral pitting; therefore scalariform lateral pitting is primitive, and this leads to the natural inference that the

tracheid type from which the vessel was derived was also scalariform.

5. A high correlation was found between the scalariform condition of the lateral walls of vessel segments and the presence of bordered pits in the fibrous elements.

6. There was some evidence to indicate that a sequence from the protoxylem to the secondary xylem would reflect the origin of the vessel.

7. Many primitive woods show, in this positional sequence, all transitions from scalariform tracheids to scalariform vessel segments.

8. The evidence would indicate that vessel segments with scalariform pitting on both the end and side walls are more primitive than vessel segments with scalariform pitting on the end walls and opposite to alternate pitting on the side walls.

9. The wood of the vessel-less angiosperms *Trochodendron*, *Tetracentron*, and *Drimys* is unquestionably very primitive. The primitive tracheids of these genera resemble, to a considerable degree, the scalariform tracheids characteristic of the primary wood of primitive angiosperms.

The writer expresses his appreciation to Dr. I. W. Bailey for many helpful suggestions, and is particularly grateful to him for the use of valuable unpublished data.

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#### LITERATURE CITED

1. BAILEY, I. W., The cambium and its derivative tissues. Amer. Jour. Bot. 7:355-367. 1920.
2. ———, Some salient lines of specialization in tracheary pitting. Ann. Botany 39:587-598. 1925.
3. BAILEY, I. W., and TUPPER, W. W., Size variation in tracheary cells. Proc. Amer. Acad. 54:150-204. 1918.
4. BAILEY, I. W., and THOMPSON, W. P., Additional notes upon the angiosperms *Tetracentron*, *Trochodendron*, and *Drimys*. Ann. Botany 32:503-512. 1918.
5. BLISS, MARY C., The vessel in seed plants. BOT. GAZ. 71:314-326. 1921.

6. BOODLE, L. A., and WORSDELL, W. C., Some points in the anatomy of Casuarinaceae and Gnetaceae. *Ann. Botany* 8:231-264. 1894.
7. BROWN, F. B. H., Scleriform pitting a primitive feature in angiospermous secondary wood. *Science N. S.* 48:16-18. 1918.
8. ———, Origin of the Hawaiian flora. *Bishop Mus., Spec. Publ.* 7:138. 1921.
9. DE BARY, A., Comparative anatomy of the phanerogams and ferns. Oxford. 1884.
10. EAMES, A. J., and MACDANIELS, L. H., An introduction to plant anatomy. New York. 1925.
11. GEGENBAUR, C., *Morphologisches Jahrbuch.* 14: p. 5. (transl. by W. B. SCOTT, The theory of evolution). New York. 1918.
12. GROOM, P., Remarks on the ecology of the Coniferae. *Ann. Botany* 24: 241-269. 1910.
13. JEFFREY, E. C., The anatomy of woody plants. Chicago. 1917.
14. JEFFREY, E. C., and COLE, R. D., Experimental investigations on the genus *Drimys*. *Ann. Botany* 30:359-368. 1916.
15. MACDUFFIE, R. C., Vessels of the gnetalean type in angiosperms. *BOT. GAZ.* 71:438-445. 1921.
16. VON MOHL, H., *Grundzüge der Anatomie und Physiologie der vegetabilischen Zelle.* Braunschweig. 1851.
17. MORGAN, T. H., A critique of the theory of evolution. Princeton. 1916.
18. SCOTT, W. B., The theory of evolution. New York. (p. 61). 1918.
19. THOMPSON, D. W., Growth and form. Cambridge. 1917.
20. THOMPSON, W. P., Independent evolution of vessels in Gnetales and angiosperms. *BOT. GAZ.* 65:83-90. 1918.
21. ———, The relationships of the different types of angiospermic vessels. *Ann. Botany* 37:183-192. 1923.
22. THOMPSON, W. P., and BAILEY, I. W., Are *Tetracentron*, *Trochodendron*, and *Drimys* specialized or primitive types. *Mem. N.Y. Bot. Gard.* 6:27-32. 1916.



# COMMON FOSSIL POLLEN OF THE ERIE BASIN<sup>1</sup>

PAUL B. SEARS

(WITH PLATES I-III)

## Introduction

This paper is intended chiefly to assist in the identification of common and significant pollen found embalmed in bog deposits in north-central United States. Before proceeding with the actual key and description, a brief explanation seems to be in order.

For a number of years European botanists have been counting the various species of pollen found at different levels in bogs. Such statistics have been used to trace the course of plant succession and the climatic changes of which it is held to be the expression. STARK (9) has prepared a concise summary on the subject up to about 1924; ERDTMAN (3) has arranged the literature preceding 1927; while FULLER (5) has twice noted for American students the general results in this field. Work continues actively in Europe and is under way in the United States.

ERDTMAN's remark that the work is not easily done is quite true. He states: "It includes field work often wet and rough, a most minute laboratory work, designing of diagrams, and study of [an extensive and widely scattered] literature." The value of such work rests upon two facts: (1) the form of pollen is, with certain reservations, definite for a species; (2) the precise structural details of pollen coats are often well preserved, not only in peat but in the silts associated with it.

Difficulties in applying these two facts arise, however, from several sources: (1) there are no comprehensive manuals of pollen structure; (2) pollen grains in bogs act as centers for flocculation of organic and inorganic materials and are not always easily loosened from this matrix; (3) it is not always possible even for an expert to distinguish related genera by their pollen, much less species of the same genus; (4) there are a number of cases of accidental resem-

<sup>1</sup> Contribution from the Botanical Laboratory, University of Oklahoma, N. S., no. 2.

blance between pollen of widely separated families; (5) not all pollen is equally well preserved, for example, it is well known that the pollen of *Juniperus* quickly breaks down; (6) some species of trees might contribute pollen quite out of proportion to nearness and abundance of the species. The phenomenon known as "sulphur showers" consists of heavy wind deposits of pine pollen, often at considerable distance from pine forests.

In spite of these difficulties, the practical results already achieved seem to justify the effort being devoted to pollen statistical analysis.

### Technique

Methods employed by European workers are discussed briefly in the paper by STARK (9), and more at length in the one by FURRER (6). The latter paper includes much critical discussion of methods and possible sources of error.

For the work done in this laboratory, samples have been secured, both with an ordinary soil auger extended by means of threaded pipe, and by means of a Davis peat sampler obtained from Eberbach & Son of Ann Arbor. This latter instrument has the advantage of supplying a continuous series of 6-inch cores to a depth of over 20 feet. The Swedish peat borer used in Europe is calibrated on a metric basis, which in some respects is an advantage. No matter what type of sampler is used it is necessary carefully to avoid contamination. In sampling a given bog attention should be paid to its profile, and samples should be taken in duplicate columns from representative portions. Samples are best preserved in glass tubes of appropriate size with corks at either end. If possible it is an advantage to mount the material soon after collecting; if not, some method of preventing bacterial action and molding should be used.

Laboratory preparation varies considerably with the character of the specimen. The principal object is to loosen the pollen grains from the colloidal substance which tends to gather about them and form an opaque mass. The usual method of accomplishing this is to place a piece of material not larger than 10 mm. in diameter in a 10 per cent solution of KOH and boil it down. An excess of glycerin is added, after which the material can be mounted, ringed, and studied. The KOH treatment has the advantage of imparting a distinct yel-

lowish to reddish stain to the grains, but it can also cause disintegration of grains unless watched carefully. Wherever the condition of the peat permits, a preferable method is deflocculation by means of ammonia water, immersing the tubes in a water bath. In such cases, by the time one is ready to add glycerin the greater portion of the alkali has volatilized. In this laboratory we have generally used glycerin-lactic acid-phenol rather than pure glycerin as a mounting medium (2), while glycerin jelly is valuable for permanent mounts. It is also often useful to presoak the specimens, either in clean distilled water or in ammonia water. Some European workers have used what seems like rather severe treatment, that is, strong acids, centrifuging, washing, etc., but with good results, notably in the case of sandy peats and interglacial material. On the whole, however, it is a safe rule to use the mildest treatment which will completely loosen all pollen from the floccules.

In mounting it is well to remove all sand particles, etc., before covering and ringing. This will permit no. 1 covers to be used, which are desirable, since oil immersion, or better, water immersion may be essential in critical identifications.

In counting, it is sound practice not to base estimates on counts of less than 100 grains. Sampling and mounting from the same sample should be done in duplicate series where possible, and care should be taken to prevent contamination by fresh pollen.<sup>2</sup> If the drops mounted are small and the pollen abundant, the euscope or similar projecting device greatly relieves the fatigue of counting. In addition to counts and percentage calculations, the pollen frequency (PF) or total number of pollen grains per square cm. of slide may be recorded; but there is no way, because of the variations of peat density, necessary treatment, etc., actually to standardize results at present.

MEINKE (8) and ERDTMAN (4), among others, distinctly emphasize the need for a comprehensive knowledge of pollen in general on the part of those attempting pollen statistical work. It is essential that the worker read WODEHOUSE's discussion of the problems of

<sup>2</sup> To realize the force of this statement, simply expose a slide rubbed with olive oil on the table during a summer day. Examination will generally reveal a considerable number of grains.

pollen structure and classification (10). The illustrated papers of ERDTMAN (4), DOKTUROWSKI (1), and MEINKE (8) deal with European species, but within certain limits they contain much useful information on American genera, occasionally on American species.

The report which follows is based upon studies of modern pollen, stained with aqueous methyl blue according to the method described by WODEHOUSE (10). The forms described were selected principally with reference to those actually found by the writer in peats; others are included on the basis of field studies in living bogs, and some on account of their abundance in the air. The literature dealing with bogs of eastern North America has also been canvassed and certain anemophilous species noted for inclusion here. The following key deals mostly with genera, and occasionally with families. Safe distinctions beyond that point, if possible at all, involve minutiae which would make the key either misleading or unwieldy. It should not be forgotten that pollen from one anther may display a considerable range of variation in size, and even in marking and form. Injuries, accidental compression, position on slide, and (in folding pollen) the degree of expansion, all have a marked effect on appearance. It is not surprising that even with the greatest care and the help of specialists there should be pollen found whose identity is uncertain. To have a certain number of unknowns in a pollen census may therefore be regarded as a mark of care rather than the reverse. To offset this situation it may be added that most of the copious producers of anemophilous pollen in the bog series have rather distinctive generic characters, difficulties being encountered mainly among the Betulaceae, Gramineae, and Cyperaceae.

#### Key to pollen

1. Pollen with two air sacs. . . . . 2  
    Pollen without air sacs. . . . . 4
2. Total length at least 100  $\mu$ . . . . . *Abies* (I, 1)<sup>3</sup>  
    Total length less than 90  $\mu$ . . . . . 3
3. Sac short-conical in lateral view, total length about 75-85  $\mu$   
    *Picea* (I, 2)  
    Sac globular or ellipsoid, total length about 75  $\mu$  or less  
    *Pinus* (I, 3, 3a)

<sup>3</sup> Roman numerals in parentheses refer to paragraphs in descriptive notes following; arabic numerals to figures in plates.

4. Pollen grains in tetrads.....5  
Pollen grains separate.....6
5. Tetrad<sup>4</sup> very compact, common wall thick, finely pitted above each grain.....*Juncus* (X, 16)  
Tetrad loose, no common wall, exine of each grain distinctly pitted and with 1 large pore (monopored).....*Typha* (IV, 7)
6. Grains without distinct perforate pores or slits (expansion folds)...7  
Grains with noticeable pores or slits (expansion folds).....12
7. Pollen subglobular to prismatic or pyramidal, exine with 1 or more inconspicuous roughened exits (germ pores)

|            |                            |
|------------|----------------------------|
| Cyperaceae | <i>Carex</i> (IX, 12)      |
|            | <i>Eriophorum</i> (IX, 13) |
|            | <i>Eleocharis</i> (IX, 14) |
|            | <i>Scirpus</i> (IX, 15)    |

- Pollen spherical or subglobular, exine with no trace of roughened exits.....8
8. Exine distinctly reticulate or papillate.....9  
Exine smooth (psilate) or finely punctate only.....10
  9. Exine reticulate, diameter 21-30  $\mu$ .....*Potamogeton* (VI, 9)  
Exine coarsely pebbled (subechinate) 60-75  $\mu$ .....*Tsuga*<sup>5</sup> (II, 6, 6a)
  10. Exine finely punctate, interrupted or broken....*Populus* (XIII, 21)  
Exine smooth (psilate) occasionally torn.....11
  11. Diameter 15-20  $\mu$ .....*Juniperus*<sup>6</sup> (II, 5)  
Diameter about 60  $\mu$ .....*Larix* (II, 4)
  12. Pores one, with or without an operculum.....13  
Pores or expansion folds more than one.....15
  13. Surface of exine smooth.....Gramineae (*Glyceria*) (VIII, 11)  
Surface of exine reticulate, pitted or pebbled.....14
  14. Surface reticulate, diameter about 20  $\mu$ .....*Typha* (IV, 7)  
Surface pebbled (subechinate).....*Sparganium* (V, 8)
  15. Pores grouped at one side of grain.....16  
Pores more or less uniformly spaced.....17
  16. Pores 10 or more aspidate or mammillate....*Juglans* (XVII, 32, 32a)  
Pores 3 simple, distinct, diameter about 50  $\mu$ .....*Carya* (XVII, 33)
  17. Pores variable but numerous, usually 10 or more.....18  
Pores fewer, usually 5 or less.....21

<sup>4</sup> *Drosera* and Ericaceae show tetrads, but of distinctly different type (MEINKE 8).

<sup>5</sup> *Osmunda* and other pteridophytes have spores somewhat similar in type, but with triradial lines.

<sup>6</sup> But *Juniperus* is seldom or never preserved for long.

18. Pores large, pollen diameter seldom less than  $25\ \mu$  ..... 19  
 Pores small, pollen diameter generally less than  $25\ \mu$   
     { *Amaranthus* (XIX, 35)  
     { *Chenopodium* (XIX, 36)
19. Surface spiny (echinate) pores irregular ..... *Sagittaria* (VII, 10)  
 Surface smooth (psilate) or nearly so ..... 20
20. Pores about 10, their margin irregular ..... *Plantago* (XXI, 38)  
 Pores about 20, with clean edges, pore lids visible in fresh  
 material ..... *Liquidambar* (XII, 18)
21. Pores 3 to 5 not in expansion folds and surface never spiny  
 (echinate) ..... 22  
 Expansion folds present ..... 25
22. Pores "aspidate" or apparently so, that is, with shieldlike or  
 mammillate thickening about them ..... 23  
 Pores not aspidate ..... 24
23. Pollen large ( $35 \times 25\ \mu$ ), compressed, punctate, pores resem-  
 bling depressions in locally thickened exine ..... *Tilia* (XI, 17, 17a)  
 Pollen smaller ( $20-30\ \mu$ ) not compressed, pores perforate  
     Betulaceae { *Betula* (XIV, 22)  
                   { *Alnus* (XIV, 23, 23a, 23b)  
     Corylaceae { *Corylus* (XIV, 24, 24a)  
                   { *Ostrya* (XIV, 25, 25a)  
                   { *Carpinus* (XIV, 26, 26a)
24. Pores 5, grain slightly pentagonal, faintly reticulate with  
 broad shallow ridges ..... *Ulmus* (XV, 29, 29a)  
 Pores 3, not projecting but sometimes with border in fresh  
 material due to thickening of intine below ..... *Celtis* (XV, 30)
25. Exine faintly reticulate or echinate ..... 26  
 Surface smooth (psilate) or at most finely pitted or papillate,  
 never reticulate or echinate ..... 31
26. Surface thickly set with sharp spines ..... *Ambrosia* (XXII, 39)  
 (spines in *Iva* not so sturdy as in *Ambrosia*) ..... *Iva*  
 Surface reticulate ..... 27
27. Pores distinct within prolonged expansion folds appearing  
 midway between apices of triangulate grain in polar view  
     *Rumex* (XX, 37)  
 Pores not distinct within ragged expansion folds or if so, grain  
 over  $40\ \mu$  in diameter ..... 28
28. Folds, usually 4, not prolonged toward poles of grain which  
 is often over  $20\ \mu$  diameter ..... *Fraxinus* (XVIII, 34, 34a)  
 Folds, usually 3, prolonged toward poles ..... 29

29. Reticulations increasing in size toward equator, periphery of grain appearing roughened. . . . . *Salix* (XIII, 20, 20a)  
 Reticulations minute, somewhat uniform, periphery of grain fairly entire. . . . . *Platanus* (XII, 19)<sup>7</sup>
30. Exine slightly thickened at edges of folds, grain compressed, about  $50 \times 40 \mu$ . . . . . *Fagus* (XIV, 27, 27a)  
 Exine not perceptibly thickened, diameter less than  $40 \mu$ . . . . . 31
31. Exine becoming very thin at margins of folds, smooth to finely rugose, grain not compressed. . . . . *Acer* (XVI, 31, 31a)  
 Exine neither thicker nor much thinner at folds, surface punctate, grains oblate spheroid. . . . . *Quercus* (XIV, 28, 28a, 28b)

### Descriptive notes

I. CONIFERS.—The pollen of *Abies*, *Picea*, and *Pinus* all have two lateral air sacs or wings, and differ principally in size, that of *Abies (balsamea)* being about  $125 \mu$  long and  $75 \mu$  high; *Picea (mariana)*  $85 \mu$  long and  $55 \mu$  high; while the pines vary  $60$ – $75 \mu$  in length and  $30$ – $35 \mu$  in height. *P. banksiana* in these samples is about  $60 \times 30 \mu$ , *P. strobus* about  $60 \times 30 \mu$ , and *P. resinosa* about  $75 \times 35 \mu$ . There are in addition slight differences in pattern and relative shape of parts, but because of the difference in position and frequent distortion of fossil material when mounted, size seems to be the safest criterion (FURRER 6). The markings (reticulations on wings, mazes and pits on central portion) are inside the coat.

II. LARIX, JUNIPERUS, THUJA.—These are similar in type, being globular without pores or distinct marking. But *Larix* is about  $65 \mu$  in diameter while the two others are considerably smaller. Of the three, *Larix* is the only one certainly found in peat, where it often retains one or more globules of its typical resinous contents. *Juniperus* and *Thuja* break down quickly when wet. *Tsuga* is an important pollen, likely to be confused with pteridophyte spores except that it lacks the triradiate prismatic faces on the inside of the latter. It is turtle-shaped, about  $70 \mu$  long with a convex warty or subechinate outer surface and a smaller, thin-walled, often sunken inner surface. Seen from above or below it of course appears globular.

III. MONOCOTYLEDONS.—Generic distinctions are often impossible, but as a rule monocotyledonous pollen of the more abundant

<sup>7</sup> May be confused with *Quercus*, *Salix*, *Fraxinus*.

kinds can be placed in the proper family, and at any rate is distinct from most dicotyledonous pollen.

IV. TYPHACEAE.—The statement is made that pollen of *Typha latifolia* is in close tetrads, that of *T. angustifolia* in linear; but the writer has found it as single grains in both. The surface is finely reticulate as in *Potamogeton*, but differs in possessing a single apical pore of moderate size. The single grain is about  $20\ \mu$  in diameter.

V. SPARGANIACEAE.—*Sparganium (eurycarpum?)* is a globular pollen of about  $25\ \mu$ , combining the monopored character of Gramineae and a granular thin surface like the exits in Cyperaceae.

VI. ZANNICHELLIACEAE.—*Potamogeton*: this group requires further study, but *P. richardsonii* shows a small elongate thick-walled grain with distinct reticulations and no discoverable pore. Dimensions  $20\text{--}25\ \mu$ . Except for smaller size of areolae and absence of pore it suggests a grain of *Typha* type.

VII. ALISMACEAE.—*Sagittaria* sp.: globularsubechinate, 10-pored, with short spiny pattern continuous over the pores. In one way this suggests the pebbly and warty exits of *Carex*, but it is perhaps more specialized, with a central spine in each pore not unlike the operculum of grasses. The several poral spines about this suggest a ragged fringe.

VIII. GRAMINEAE.—As pointed out by WODEHOUSE (10), generic distinctions are difficult in this family, but the family character is unmistakable. Pollen globular to ovate, varying greatly in size but all monopored psilate (1-pored, smooth). The distinct central operculum may be absent in fossil material.

IX. CYPERACEAE.—The form of pollen in this family may vary from globular to pyramidal. The exine character however is unmistakable. At one end is a thin-walled warty or pebbly area, and in all forms examined (*Carex*, *Scirpus*, *Eleocharis*, *Eriophorum*) there are from one to three similar areas elsewhere on the exine, harder to distinguish than that of the end. In consulting the key and drawings it should be remembered that further work needs to be done on this difficult group before determinations can safely be made. The outline drawings of MEINKE (8) should be consulted if possible.

X. JUNCACEAE.—This group has not been studied adequately, but *Juncus effusus* shows a tight tetrad with a thick more or less hyaline



wall and a faintly broken area as in *Carex*, above each of the four protoplasts.

XI. DICOTYLEDONS.—Tiliales: the pollen of *Tilia* (*americana* or *europaea*) is very typical. It is flattened, triangulate, with three median pores, each appearing like a pit in the locally thickened exine. Surface of exine finely pitted. Diameters about  $35 \times 25 \mu$ .

XII. HAMAMELIDALES.—The two genera *Liquidambar* and *Platanus* are in different families and possess distinctly different types of pollen. That of *Liquidambar* has about ten pores with lids, no expansion folds, is about  $35 \mu$  in diameter, with nearly smooth surface. In fossil material with lids gone it might be confused with chenopodiaceous pollen except for its larger size. Pollen of *Platanus* is tricolpate, finely reticulate, and about  $20 \mu$  in diameter. It may be confused with pollen of *Quercus* or *Salix*. It appears to show flecks of exine over the furrowed surface, which may be homologous to the flecked lids in *Liquidambar*.

XIII. SALICALES.—*Populus* has a very simple globoid pollen with a thin granular interrupted exine, and no pores. It is distinguished from *Juniperus* by its larger diameter (about  $30 \mu$ ) and exine character. *Salix* has a tricolpate grain ( $20 \mu$  in diameter) with small reticulations at the poles and larger ones at the equators. Folds often appear median in polar view (cf. especially *Fraxinus* and *Platanus*).

XIV. FAGALES.—The pollen of American forms of this order require critical study because of their extreme importance in pollen analysis. For European forms see JENTYS-SZAFER (7). The Betulaceae (*Betula*, *Alnus*) and Corylaceae (*Corylus*, *Ostrya*, *Carpinus*) are readily distinguished from the Fagaceae (*Fagus*, *Quercus*) by the mammillate or blister-like thickened exine at each pore ("aspidate"<sup>8</sup>) in the first two families, and the presence of three expansion folds in the last. Beyond these limits, however, determination of genera is difficult. The present key makes no attempt to separate the Betulaceae from the Corylaceae, or to distinguish their genera. The pollen of *Fagus* ( $50 \times 40 \mu$ ) is larger than that of *Quercus* ( $30 \times 20 \mu$ ), but both are, unlike *Acer*, compressed at the poles. *Fagus* often shows distinct thickening of the exine along the expansion fold, while that of *Quercus* does not. It should be noted that the

<sup>8</sup> WODEHOUSE, unpublished.

aspidate character is marked in *Juglans* and perhaps suggested in *Celtis*, but other characters make the distinction of these genera from *Betula*, etc., possible.

XV. URTICALES.—*Ulmus* is a distinctly pentagonal grain of about  $30\ \mu$  diameter in polar view, but appears ovoid in lateral view, being flattened. There are five oval pores, one at each angle of the equatorial line, and the surface is distinctly undulate because of large shallow areolae. Exine is thickened about the pores. *Celtis* likewise has equatorial pores, but only three and the grain is quite globular. It is about  $30\ \mu$  in diameter and may appear aspidate when fresh, due to a lenslike thickening of intine below the pore.

XVI. SAPINDALES.—*Acer saccharum* ( $32\text{--}38\ \mu$ ) would be confused with the tricolpate (three-furrowed) oak, except for its globular form, finely striated surface, and the fact that its exine becomes extremely thin at the edges of the folds. This last character seems to hold in all species of *Acer* examined. It must be remembered that there is a great mass of (at present) nondescript tricolpate pollens of herbaceous and shrubby plants, but fortunately few are abundantly wind-borne.

XVII. JUGLANDALES.—In *Carya* and *Juglans* the distribution of pores (three in *Carya*, ten or more in *Juglans*) is asymmetrical. The pores of *Juglans* are distinctly aspidate, making this pollen (of  $35\times 30\ \mu$ ) easy to determine. Those of *Carya* appear as plain perforations, lying on a smaller circle than the equator of the very large grain ( $50\text{--}55\ \mu$ ).

XVIII. LOGANIALES.—The pollen of *Fraxinus* is very similar to that of *Salix* and *Platanus*, but is usually quadrangulate oblate, and the four (or three) apical expansion furrows of the reticulate grain are short. Dimensions about  $25\times 20\ \mu$ .

XIX. CHENOPODIALES.—The pollen of this order is well known and distinctive, being globular, psilate (smooth), without expansion folds but with numerous perforate pores each having a basal membrane faintly flecked. The pore number varies so much within genera that no attempt can be made to separate Chenopodiaceae and Amarantaceae.

XX. POLYGONALES.—*Rumex* is of the familiar tricolpate type, but there is a distinct equatorial pore in each fold. The folds lie between, not at, the blunt angles of the equator and are long. Exine reticulate; diameter about  $25\ \mu$ .

XXI. PLANTAGINALES.—*Plantago* is abundant in modern air and resembles *Sagittaria* (q.v.) in size (25–30  $\mu$ ), number (ten), and ragged margins of pores, but is smooth instead of spiny.

XXII. ASTERALES.—Composite pollen is thick-walled, tricolpate, but differs greatly in details of pattern. *Ambrosia* and perhaps *Iva* are the two most likely to be of interest in anemophilous deposits. General characters are clear from key and figure, but further information should be secured, if desired, from the papers of WODEHOUSE, especially (10).

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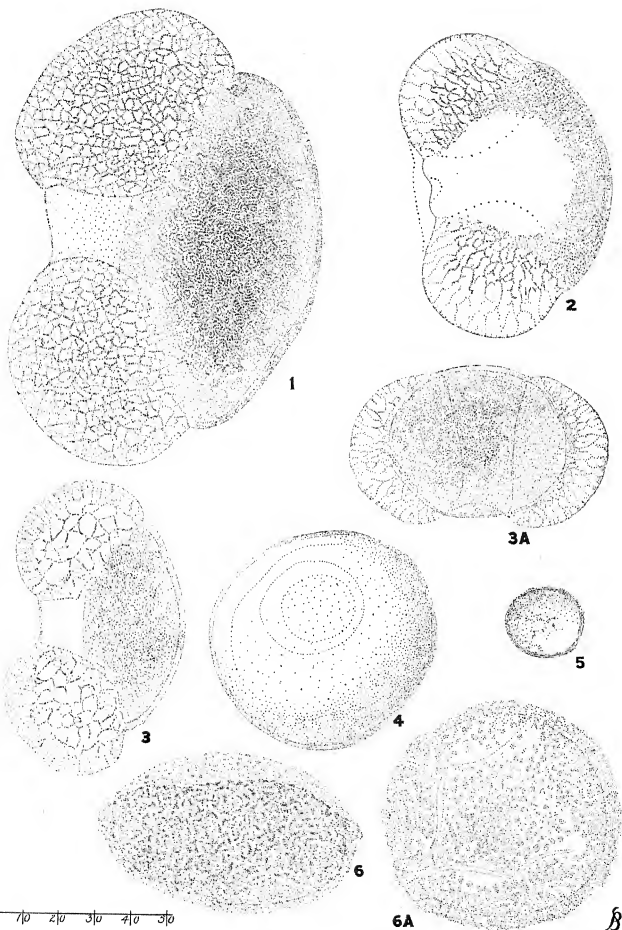
#### LITERATURE CITED

1. DOKTUROWSKI, W., Pollen im Torf. Mitt. wissensch.-experiment. Torfinstitut Moskau. (Russ.) no. 5. 33–44. 1923.
2. DRAFER, P. I., A demonstration of the technique of pollen analysis. Proc. Okla. Acad. Sci. 8:63–64. 1928.
3. ERDTMAN, G., Literature on pollen statistics published before 1927. Geol. Förf. I. Stockholm Förf. 49:196–211. 1927.
4. ———, Beitrag zur Kenntniss der Mikrofossilien. Ark. Bot. 18:1–9. 1923.
5. FULLER, G. D., Peat bogs and postglacial vegetation. BOT. GAZ. 87:560–562. 1929 (also *ibid.* 83:323–325. 1927).
6. FURRER, E., Pollenanalytische Studien in der Schweiz. Beibl. Vierteljahrsschr. Naturf. Gesell. in Zürich. 72:1–38. 1927.
7. JENTYS-SZAFER, J., La structure des membranes du pollen de *Corylus*, de *Myrica*. Bull. Acad. Pol. Sci. Lett. Cl. Sc. Math.-Nat. B. Bot. 75–125. 1928.
8. MEINKE, H., Atlas und Bestimmungsschlüssel zur Pollenanalytik. Bot. Arch. 19:380–449. 1927.
9. STARK, P., Der gegenwärtige Stand der pollenanalytischen Forschung (Sammelreferat). Zeitschr. Bot. 17:89–125. 1925.
10. WODEHOUSE, R. P., The phylogenetic value of pollen grain characters. Ann. Botany 42:891–934. 1928.

## EXPLANATION OF PLATES I-III

All figures were drawn with camera lucida and Leitz microscope; details drawn under oil immersion where necessary. As reproduced all represent a magnification of 700 diameters, except fig. 27, which is about 450.

- FIG. 1.—*Abies balsamea*, lateral view.  
FIG. 2.—*Picea mariana*, lateral view, showing subconical air sacs.  
FIG. 3.—*Pinus strobus*, lateral view; fig. 3a, top view.  
FIG. 4.—*Larix decidua*, showing globule of resinous contents.  
FIG. 5.—*Juniperus virginiana*; *Thuja* very similar.  
FIG. 6.—*Tsuga canadensis*, lateral view (ventral side up) showing coarse projecting dorsal coat and thin ventral one; fig. 6a, ventral view.  
FIG. 7.—*Typha latifolia*, typical close tetrad, but grains often occur singly.  
FIG. 8.—*Sparganium* sp. showing single roughly operculate pore and pebbly surface.  
FIG. 9.—*Potamogeton richardsonii*.  
FIG. 10.—*Sagittaria* sp.  
FIG. 11.—*Glyceria borealis*, single operculate pore and smooth surface.  
FIG. 12.—*Carex vulpinoides*, showing, with the next three, typical granular exits of Cyperaceae.  
FIG. 13.—*Eriophorum virginicum*.  
FIG. 14.—*Eleocharis palustris*.  
FIG. 15.—*Scirpus americanus*.  
FIG. 16.—*Juncus effusus*.  
FIG. 17.—*Tilia americana*, polar view; fig. 17a, equatorial view.  
FIG. 18.—*Liquidambar styraciflua*.  
FIG. 19.—*Platanus occidentalis*.  
FIG. 20, 20a.—*Salix sericea*.  
FIG. 21.—*Populus deltoides*.  
FIG. 22.—*Betula lutea*.  
FIG. 23, 23a, 23b.—*Alnus incana*.  
FIG. 24, 24a.—*Corylus americana*.  
FIG. 25, 25a.—*Ostrya virginiana*.  
FIG. 26, 26a.—*Carpinus caroliniana*.  
FIG. 27.—*Fagus grandifolia*, polar view ( $\times 450$ ); fig. 27a, equatorial view ( $\times 700$ ).  
FIG. 28.—*Quercus alba*; fig. 28a, *Q. macrocarpa*; fig. 28b, *Q. rubra*.  
FIG. 29, 29a.—*Ulmus americana*.  
FIG. 30.—*Celtis occidentalis*.  
FIG. 31, 31a.—*Acer saccharum*.  
FIG. 32, 32a.—*Juglans nigra*.  
FIG. 33.—*Carya ovata*, polar view.  
FIG. 34, 34a.—*Fraxinus lanceolata*.  
FIG. 35.—*Amaranthus retroflexus*.  
FIG. 36.—*Chenopodium* sp.  
FIG. 37.—*Rumex brittanica*.  
FIG. 38.—*Plantago rugelii*.  
FIG. 39.—*Ambrosia psilostachya*.







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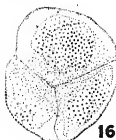
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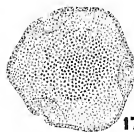
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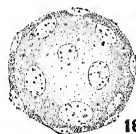
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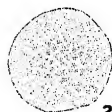
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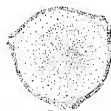
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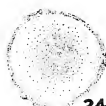
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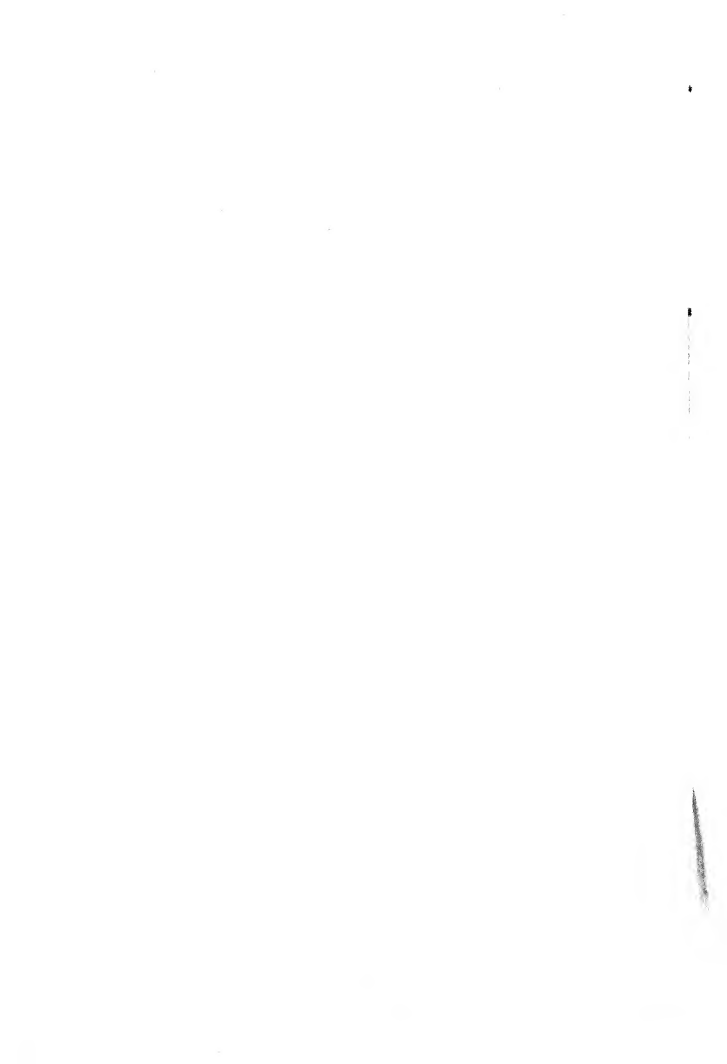
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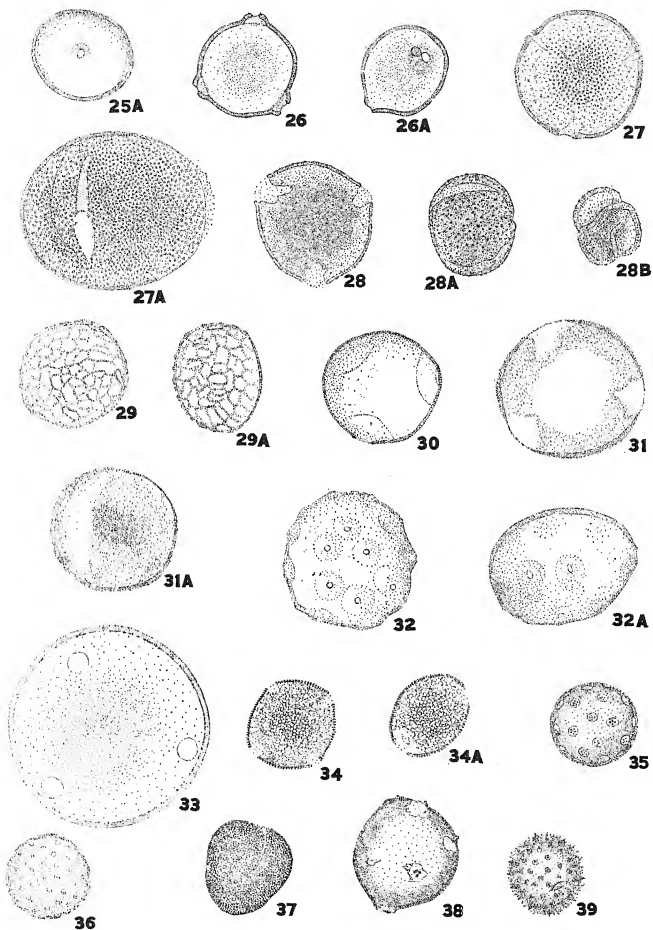
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# CURRENT LITERATURE

## BOOK REVIEWS

### New books on paleobotany

The two manuals here reviewed serve entirely different purposes. The French book<sup>1</sup> is a classroom syllabus. The German text,<sup>2</sup> of which only the first volume has appeared, is a large reference book which intends to summarize our present knowledge of fossil plant structure, in two profusely illustrated volumes. The first book is a great help in teaching paleobotany and the other one is indispensable for research. There is no up-to-date English textbook for a general introduction to paleobotany, and the literature on this subject is evenly divided between the three languages English, German, and French. No one can make a thorough study of paleobotany without a fluent command of these three languages. Of late a large number of botanical textbooks have been published in German, including some on paleobotany.<sup>3</sup>

A handy outline of paleobotany for American students was published by BERRY<sup>4</sup> in 1920, but is out of print now because it appeared in a government publication. The standard English textbook for students of fossil plant structures is, and will be for a long time, SCOTT's work,<sup>5</sup> while the great reference book by Seward<sup>6</sup> attempts to give a complete account of fossil pteridophytes and gymnosperms, devoting only a small space to thallophytes and bryophytes. Some portions of SEWARD's work are out of date, and the four volumes do not contain enough illustrations to fulfill the object of being a complete reference book.

HIRMER's handbook compares favorably with the volumes by SCOTT and SEWARD, on account of the very large number of its well executed illustrations, and also because of its very complete bibliography. HIRMER attempts many

<sup>1</sup> BERTRAND, P., *Conférences de Paléobotanique*. 8vo. pp. 138. Librairie de l'enseignement technique, 3 Rue Thenard, Paris. Léon Eyrolles, Éditeur. 1926.

<sup>2</sup> HIRMER, M., *Handbuch der Paläobotanik* (with chapters by JULIUS PIA and WILHELM TROLL). Vol. I. Thallophyta, Bryophyta, Pteridophyta. 8vo. pp. xvi+708. figs. 817. Munich and Berlin: R. Oldenbourg. 1927.

<sup>3</sup> POTONIÉ, H., *Lehrbuch der Paläobotanik*. 2d ed. Berlin. 1921. GOTHAN, W., *Paläobotanik*. Berlin and Leipzig: Sammlung Göschen. 1920.

<sup>4</sup> BERRY, E. W., *Paleobotany: a sketch of the origin and evolution of floras*. Smithsonian Report for 1918. pp. 289-407. Washington. 1920.

<sup>5</sup> SCOTT, D. H., *Studies in fossil botany*. 2 vols. 3d ed. London. 1920-23.

<sup>6</sup> SEWARD, A. C., *Fossil plants*. 4 vols. Cambridge. 1898-1919.

reconstructions, and devotes considerable attention to the classification and organization of his material. His book is up-to-date and should now be used as a basis for any research undertaken in fossil plant structures. It is to be hoped that the second volume, which will contain the gymnosperms, will appear soon.—A. C. NOÉ.

### Organography of plants

The appearance of a new edition of GOEBEL's principal book<sup>7</sup> in which his morphological life work is summarized, is an historical event in botanical literature.

This new edition is considerably enlarged. The number of illustrations has been increased from 459 in the second edition to 621 in the third edition of part I; and there are about 100 more pages. The second edition appeared in 1913.

A. P. DECANDOLLE designated as the subject of his "*Organographie végétale*" (Paris 1827) the "*Description raisonnée des organes*," and he also included the anatomy. The latter is excluded for practical reasons in GOEBEL's organography. GOEBEL distinguishes two problems in his organography; the first one is to discover how the many different forms of plant organs originate and what the relations between them are. GOEBEL gave the name morphology to this problem. The second problem of organography is, according to GOEBEL's the investigation of the connection between plant forms and living conditions.

Part I deals with general organography. It considers the formation of organs in the different levels of the plant kingdom, with the problem of symmetry in the plant, the transformation, reduction, fusion, and partition of plant organs. In the last chapter the author discusses the relation of the formation of organs to internal and external factors.

GOEBEL's conception of organography forms a very interesting section of botanical science. It is a cross-section through several divisions of standardized botany, and connects a great many facts which are customarily distributed among our conventional divisions morphology, physiology, and ecology. Probably its main value lies in the fact that plant life is studied in this volume from a different angle from that found represented in conventional classification of plant sciences.—A. C. NOÉ.

### Fungi of North America

A very noteworthy contribution has been made by SEYMOUR,<sup>8</sup> in his monumental compilation of the fungi which have been reported to occur on North American hosts. It is fitting that this volume grew out of the early manuscripts of FARLOW'S, which were used in his edition of 1888-1891; that it is the result

<sup>7</sup> GOEBEL, K., *Organographie der Pflanzen*. Part I. *Allgemeine Organographie*. 8vo. pp. ix+642. figs. 621. Jena: Gustav Fischer. 1928.

<sup>8</sup> SEYMOUR, A. B., *Host index of the fungi of North America*. pp. xiii+732. Cambridge: Harvard University Press. 1929.

of the labors of his associate through all the years; that it was made possible by the interest and material assistance of the late Mrs. FARLOW; and that it is published by the Harvard University Press.

The volume contains 80,000 names of hosts and fungi. Since all systems are arbitrary, it is to be hoped that the system used here will tend to stabilize host and parasite names in the United States. An attempt is made to apply to fungi the International Rules of Nomenclature adopted at Brussels, which may not meet with entire approval by all systematists.

The volume will prove an invaluable addition to the library of every mycologist and phytopathologist. It is well printed on high grade paper.—G. K. K. LINK.

### Mycorrhiza

Biology is benefited by Miss RAYNER's publication as a separate volume<sup>9</sup> of her papers on mycorrhiza which appeared in the New Phytologist. This volume is of special appeal to those interested in plant nutrition, infection, and adaptation. Although the volume is essentially a review, it is more than a mere compilation, in that the material is critically evaluated. The author states in conclusion: "On the whole it may be said that the views so strongly held by FRANK, STAHL, and their followers have survived the test of experimental inquiry. . . . In the opinion of the writer, there can be no doubt that recent investigations by means of pure cultures have tended to support the view that possession of mycorrhiza is frequently of benefit to the vascular hosts."

The first chapter is devoted to the early period of mycorrhizal studies; the second and third to the second period, 1880-1900; the next six chapters to the modern period; the last two chapters to discussion of tuberization and the physiological significance of mycorrhiza.—G. K. K. LINK.

### Basidiomycetes

A volume by ULBRICH<sup>10</sup> is a revision in the third edition of *Die höheren Pilze* (Basidiomycetes), which is the first volume in the excellent series *Kryptogamen Flora für Anfänger*, inaugurated by the late Professor LINDAU. In harmony with the results of recent research, radical changes were made in the general and in the special parts and keys, so that the presentation is in line with modern concepts and interpretations relative to structure, life histories, and cytology of Basidiomycetes. Although this volume is designed for the use of beginners and amateurs in central Europe, it will be found of considerable use by students of the Basidiomycetes in all parts of the world.—G. K. K. LINK.

<sup>9</sup> RAYNER, M. C., Mycorrhiza, an account of non-pathogenic infection by fungi in vascular plants and bryophytes. New Phytol. Reprint no. 15. pp. vi+246. figs. 64. pls. 7. London: Wheldon and Wesley, Ltd. 1927.

<sup>10</sup> ULBRICH, E., Die höheren Pilze. Basidiomycetes. Frontispiece portrait of Lindau. xii+497. figs. 38. 14 plates in supplement. Julius Springer. Berlin. 1928.

### Paleoecology

A report of the ecological conditions which prevailed during a part of the Permian age in central Germany has been published by WEIGELT.<sup>11</sup> The larger portion of the book is devoted to a description of species, while only a comparatively small part of it deals with the ecological conditions of the period and with other general questions.

The period of the "Kupferschiefers" or copper slate is situated in the lower Zechstein, which, in turn, represents the upper Permian of central Europe. These deposits of copper slate originated in a sea which may have had some similarity to the Black Sea of our age. The shores of this Permian Sea were lined with a vegetation which indicates climatic contrasts with strong insulation and abrupt changes in temperature during which the danger of drought was present.

This vegetation consisted primarily of conifers, Cycadofilicales, ferns, and Equisetales. These plants were characterized by heterophylly, and the author draws interesting conclusions on climatic conditions from this fact. Heterophylly, in our present-day flora, often indicates that the plant organism grows partly in one condition and partly in another. When a plant grows out from the shade into the sunlight, or from moist surroundings into drier ones, we may obtain heterophylly. The conclusions which the author draws from present conditions upon those of the past are very enlightening.—A. C. NOÉ.

### Protoplasm

An international periodical on protoplasm<sup>12</sup> was started in July, 1926, devoted to the study of the physical chemistry of protoplasm. The subtitle of the periodical is given in four languages, German, English, French, and Italian. Original articles may be written in any one of these four languages. The purpose of the journal is to unite and to harmonize such sciences as deal with the physical chemistry of protoplasm for the benefit of investigators whose fields are now separated by barriers, but who are all interested in the results of protoplasm research. Among the subjects which the periodical aims to cultivate are colloidal chemistry and microchemistry of protoplasm, protoplasm structure, osmosis, plasmolysis and cytolysis of protoplasm, ultra microscopy as applicable to protoplasm research, the mechanics of cellular and nuclear division; and the pathology of protoplasts. The editors hope that physiologists, cytologists, pathologists, and histologists will be equally interested in this periodical.—A. C. NOÉ.

<sup>11</sup> WEIGELT, J., *Die Pflanzenreste des mitteldeutschen Kupferschiefers und ihre Einschaltung ins Sediment. Eine palökologische Studie.* pp. iv+395-592. pls. 35. figs. 14. Berlin: Gebrüder Borntraeger. 1928.

<sup>12</sup> *Protoplasma*, edited by J. SPEK (Heidelberg) and J. WEBER (Graz) in collaboration with R. CHAMBERS (New York) and W. SEIFRIZ (Philadelphia). Berlin: Gebrüder Borntraeger.

### Plant geography

The first series of monographs in plant geography<sup>13</sup> published by FISCHER in Jena has been completed. Each number contains the monographic treatment of several species, genera, or even families of plants, and is well supplied with maps. There are usually ten maps to a number, and 10-16 pages of text. Each plant type is treated by a competent author, and the names of the best German plant taxonomists are represented on the list. The publishers call this series an "Archiv," in which maps of the natural distribution of all plant groups will be collected and explained. The main object is to give diagrammatic maps of the distribution of recent and fossil plants in order to supply a basis for floristic analysis, plant distribution, and the evolution of floral areas. Also, certain phylogenetic problems of theoretic taxonomy are to be explained by these maps. It is not to be expected that the series can be completed in a few years, and therefore the character of this publication is bound to be that of a periodical.—A. C. NOÉ.

### Anatomy of monocotyledons

A very important anatomical study of the monocotyledons<sup>14</sup> is more than half completed. It is planned to comprise seven volumes, with a total of about 800 pages. Four of these volumes have already appeared. The first one treated Pandanales, Helobiae, Triuridales; the second, the Glumiflorae; the third, the Principes, Synanthae, Spathiflorae; and the fourth one, which has recently appeared, the Farinosae. The fifth volume will discuss Liliiflorae; the sixth one Scitamineae and Microspermeae; and the seventh will contain a discussion of the monocotyledons in general and an index for the entire publication. The individual volumes are being published at intervals of about six months, and the entire publication is intended to be an exhaustive anatomical reference book, with special consideration of the taxonomy of the monocotyledons.—A. C. NOÉ.

### Reference book of plant anatomy

The largest existing reference book on plant anatomy is being edited by K. LINSBAUER,<sup>15</sup> of Graz, Austria. A number of European scholars from different countries are cooperating in the publication of this monumental work, which is intended to comprise our present-day knowledge of plant tissues. It is still impossible to say how many volumes will appear and when the entire publica-

<sup>13</sup> Die Pflanzenareale, edited by E. HANNIG and H. WINKLER, in collaboration with L. DIELS and G. SAMUELSSON. 1st series. nos. 1-8. 4to. Jena: Gustav Fischer. 1926-1928.

<sup>14</sup> SOLEREDER, H., and MEYER, F. J., Systematische Anatomie der Monokotyledonen. Vol. IV. Farinosae. 8vo. pp. 176. figs. 65. Berlin: Gebrüder Borntraeger. 1929.

<sup>15</sup> Handbuch der Pflanzenanatomie, edited by K. LINSBAUER. 8vo. Berlin: Gebrüder Borntraeger. 1922.

tion will be completed. Vols. I-X have appeared to date, either in part or in full, and they show how thoroughly the subject of plant anatomy is to be covered. The entire literature is considered, to judge from the bibliography quoted in this volume. It is to be hoped that this very important publication will soon be completed.—A. C. NOÉ.

#### NOTES FOR STUDENTS

**New stem type from Devonian rocks of Australia.**—A new Devonian stem has been found in the Burdekin beds, Burdekin basin, Queensland, and sent to SEWARD of Cambridge University, England, for examination. He turned it over to HARRIS for examination and description.<sup>16</sup> It is of special interest that the stem is from the Middle Devonian, and therefore throws light upon the divertment of the stele. Every contribution to our knowledge of Devonian plant structures is helpful, and it is fortunate that in the past few years a number of Devonian plant structures have been described.

The most interesting comparison of *Schizopodium davidi* is with *Asteroxylon*, which was first described by KIDSTON and LANG, and later by KRÄUSEL and WEYLAND. The main differences between the two genera can be summarized in the following statement: the new genus has no leaves, but has on the other hand a sort of secondary wood. Also the stele in *Schizopodium* has the shape of the xylem, which is commonly a star, while in *Asteroxylon* the stele is round, even when the xylem is star-shaped.—A. C. NOÉ.

**An interesting plant hybrid.**—The raising of hybrids between sugar-cane and cholam (*Andropogon sorghum*) has just been successfully accomplished by Mr. RAO BAHADUR T. S. VENKATRAMAN,<sup>17</sup> Imperial Cane Breeding Station, Coimbatore. As an intergeneric hybrid (by no means too common in the vegetable kingdom) this should prove of considerable scientific interest. The three such hybrids of possible economic value known at present are the "maize-teosinte," the "wheat-rye," and the "cabbage-radish" hybrids. The F<sub>1</sub> generation of sugar-cane being noted for diversity among the progeny, and sugar-cane, as a crop, being propagated vegetatively, it is not impossible that important economic results might come from a "cane-cholam" hybrid.

<sup>16</sup> HARRIS, T. M., *Schizopodium davidi* gen. et. sp. nov.: a new type of stem from the Devonian rocks of Australia. Phil Trans. Royal Soc. London. Series B 217: 395-410. pls. 91-93. London. 1929.

<sup>17</sup> Madras Agric. Jour. 17: no. 11. p. 359. 1929.



# THE BOTANICAL GAZETTE

April 1930

## EFFECTS OF ULTRA-VIOLET RADIATION UPON SPORULATION IN MACROSPORIUM AND FUSARIUM<sup>1</sup>

G. B. RAMSEY AND ALICE ALLEN BAILEY<sup>2</sup>

(WITH PLATE IV AND TWELVE FIGURES)

### Introduction

In taxonomic studies of some strains and species of *Macrosporium* and *Alternaria* pathogenic to tomatoes, the difficulty of obtaining spores in artificial culture was encountered. The same problem arose in studies of members of the genus *Fusarium* causing bulb rot of onions. Many strains of *Fusarium* produced microspores in abundance, but failed to produce the macrospores which are necessary for classification of the species. With each genus various methods of obtaining sporulation were tried, such as varying the moisture content of the medium or the kind of medium employed, varying the hydrogen-ion concentration, changing the relative humidity of the air to which the fungus was exposed, varying the temperature, and mutilating the mycelium as recommended by RANDS (8) and KUNKEL (7). Small quantities of spores were obtained at times with these methods, but with no consistency, so that duplication of results could not be relied upon.

<sup>1</sup> Contribution from the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and the Department of Botany, University of Chicago, cooperating.

<sup>2</sup> The writers are greatly indebted to Professor CHARLES A. SHULL of the Department of Botany for use of the quartz mercury arc, and to Dr. H. B. LEMON and his students in the Department of Physics for use of apparatus and the making of spectrograms.

Field observations have shown that *Macrosporium* and *Alternaria* species affecting tomatoes usually sporulate quite freely on the plants. These fungi produced spores very rarely in pure culture in the laboratory, although good vegetative growth was readily obtained by the usual methods of culturing and under a wide variety of conditions. Since spores were not obtained, a search for the factors favoring sporulation in nature led to a consideration of the effects of light. The fact that sunlight in the laboratory failed to induce sporulation suggested the possibility that it might be the short rays toward the ultra-violet region of the spectrum which were effective. Most of these are excluded by window glass and by the culture vessels. Hence, studies of the effect of ultra-violet radiation upon sporulation in fungi were initiated in November, 1927, at which time a review of the literature failed to reveal any work covering this particular type of reaction to ultra-violet radiation. The results obtained were so favorable (fig. 1) that it was decided to continue the investigations.

Most of the previous investigations of the effects of ultra-violet radiations upon fungi have been concerned with determining the fungicidal action of these rays. BOVIE (1) found that bacteria and spores of various fungi were killed by a 10-minute exposure to wave lengths shorter than 2925 Ångstrom units. With wave lengths longer than this the organisms were not killed by an exposure of 120 minutes. A change of only 25 m $\mu$  was sufficient to cause this change in the killing power of light. In 1916 BOVIE (2), working with the Schumann rays (1250-2000 A.u.), reported that exposures which were lethal to hyaline spores were not lethal to such dark-colored spores as those of *Stemphyllium*. He explained the resistance of the dark-colored spores on the ground that the Schumann rays have not sufficient penetrating power to pass through the colored cell walls. Pigmented yeasts are similarly more resistant to ultra-violet than white yeasts, according to the work of TANNER and RYDER (12), who found that yeast was killed by the short rays although it lived longer than bacteria. Their data indicated a direct relation between size of the cell and its resistance to ultra-violet rays. CHAVARRIA and CLARK (4) also cited pigmentation as a defensive mechanism against lethal action. They found that visible and near ultra-violet light

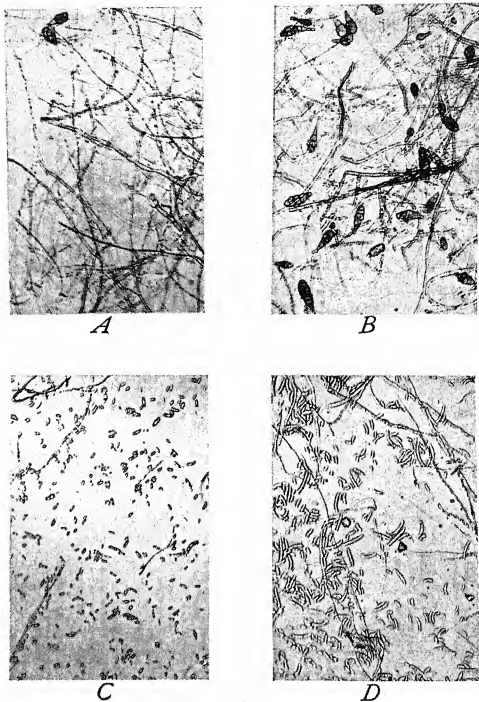


FIG. 1.—A, scarcity of spores in control culture of *Macrosporium tomato*; B, spores of same induced by a 15-minute exposure to quartz mercury arc through vitaglass at 40 cm.; C, scarcity of macrospores in control culture of *Fusarium cepae*; D, macrospores of *Fusarium cepae* induced by a 15-minute exposure to quartz mercury arc through vitaglass at 40 cm.

together were stimulating in moderate doses to pathogenic fungi of the skin, but had a slightly inhibitory effect in heavy doses in non-pigmented fungi.

Very short exposures are effective in producing marked change in some organisms. An irradiation of two or three minutes at a distance of 15 cm. from a mercury arc is sufficient to cause cytolysis in zoospores of *Blepharospora* placed in a thin film of water (DUFRENOY 5). Even shorter exposures are sufficient to kill the spores of *Fusarium echinosporum* and *F. fuliginosporum*, according to SIBILIA (9).

In recent studies of the fungicidal action of ultra-violet radiations, FULTON and COBLENTZ (6) found that the spores of the twenty-seven miscellaneous species of fungi under observation were killed in most instances by a comparatively short exposure to ultra-violet radiations. The different wave-length components showed different killing power, which increased as the wave length decreased, the lethal action beginning at about 365  $m\mu$  as an upper limit.

In addition to a lethal effect on the aerial mycelium and spores of *Glomerella cingulata*, STEVENS (10, 11) reported a stimulation of production of acervuli and perithecia when the fungus was exposed to ultra-violet rays. He found that direct radiation from a quartz mercury arc for periods of 0.25 to 4 seconds resulted in the production of surface perithecia in agar colonies, and that longer dosage (5 seconds to 2 minutes) resulted in buried perithecia. Colonies as young as two days produced perithecia, but not so many as were produced on colonies 4 days old. The efficient rays of the ultra-violet were found to be throughout the region with wave lengths shorter than 313  $m\mu$ . *Coniothyrium* sp. was induced to produce pycnidia earlier than normal by an irradiation of 10 seconds.

### Investigation

The present work is not concerned with the lethal effects of the ultra-violet rays but is confined to a study of the production of spores as a result of exposure. In all experiments a Cooper-Hewitt quartz mercury arc lamp was used, operated on direct current at 4 amperes through resistance from a 110 volt line. Exposures were made at a distance of 40 cm. from the arc unless otherwise stated.

In the first experiments lids for petri dishes were made by molding

paraffin rings on squares of vitaglass.<sup>3</sup> These were sterilized with alcohol and allowed to dry before substituting them for the regular petri dish lid, previous to exposure. Plantings of the fungi were made on potato-dextrose agar, and they were allowed to grow until the colonies attained a diameter of approximately 2.5 cm. before irradiation was begun. In all experiments several plates were used for each exposure and the average reading of many mounts obtained. The results were recorded in terms of percentage of macrospores in *Fusarium*, and in number of spores per low power field of the microscope in *Macrosporium*. Controls were held for an equal length of time in the diffused light of the laboratory at room temperature. In preliminary experiments exposures were made for varying periods of time from one minute up to one hour. Irradiations were given for six successive days and spore counts were made on the seventh day.

Although sufficient preliminary tests have been made with several strains of *Macrosporium* and species of *Fusarium* to show that the treatment may have quite wide application, the present discussion is confined to the work with the fungus causing Nailhead Spot of tomatoes, *Macrosporium tomato* CKe.<sup>4</sup> and *Fusarium cepae* Hanz emend Link & Bailey. Detailed studies were made of these two fungi at this time.

The results of a typical experiment with *Macrosporium tomato* are shown in fig. 2. It will be noted that even with the 1-minute exposure at 60 cm. under vitaglass the average number of spores per low power field was about twelve times that of the control. The number of spores increased with the increase in length of exposure, the 60-minute plates having forty-four times as many spores as the controls. In fig. 3 the results of a similar experiment with *Fusarium cepae* show that the percentage of macrospores increased with the length of exposure. Counts of many fields showed that an exposure of one minute gave an average of 12 per cent macrospores as compared with the 5 per cent in the control, while an exposure of one hour increased the macrospores to 65 per cent. Although the control plates showed no exposed pionnotes or sporodochia, but a sea-shell

<sup>3</sup> Vitaglass furnished by courtesy of Vitaglass Corporation, New York City.

<sup>4</sup> According to ROSENBAUM (Phytopath. 10:9-22, 1920). The writers find this fungus produces spores in long chains in a humid atmosphere.

pink<sup>5</sup> to pale salmon-colored fluffy mycelium, in plates exposed five minutes there was an exposed pionnotes over 2 cm. in diameter with small salmon-colored sporodochia under the mycelium beyond the pionnotes. The cultures exposed one hour showed no aerial mycelium but salmon-colored pionnotes covered the plate. Irradiation through

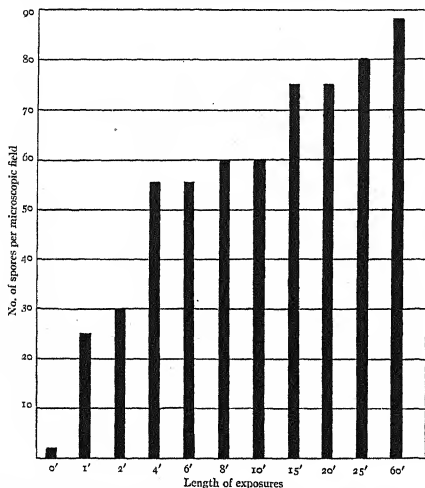


FIG. 2.—Effect on spore production of exposing *Macrosporium tomato* cultures at 60 cm. from quartz mercury arc through vitaglass for various periods of time on six successive days.

vitaglass produced a slight diminution in growth rate (figs. 13 A, C; 14). The controls showed an average daily increase in diameter of 6 mm., whereas the 1-minute plates showed an average increase of 5.25 mm., the 5-minute plates 4.75 mm., the 10- and 20-minute plates 4.5 mm., and the hour plates 4.3 mm. No abnormal or disrupted spores occurred.

<sup>5</sup> RIDGWAY, R., Color standards and color nomenclature. Washington, D.C. 1912.

Check experiments were run to determine whether heat produced by the arc might be the factor responsible for inducing spore production. Plates were inoculated as before and, except for one hour a day, were kept with control cultures in diffuse light in the laboratory, at approximately  $22^{\circ}$  C. Exposures were for one hour at 60 cm. from the arc on six successive days. The results obtained from these experiments are given in table I, which clearly shows that temperature is not the important factor in determining sporulation.

Change in the medium due to irradiation was eliminated as a causal factor of increased sporulation by the following experiment. Duplicate sets of plates of potato dextrose agar were poured and covered with vitaglass. One set of these was irradiated before inoculation with the fungi, two plates for 15 minutes, two for 30 minutes, and two for one hour each day. After the third irradiation these plates and the duplicate unirradiated plates were all inoculated with *Macrosporium*. The test was also duplicated for *Fusarium*. After the

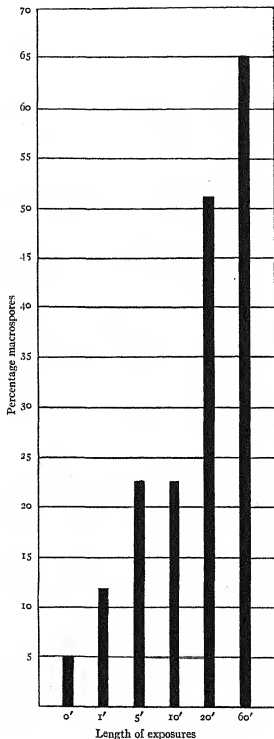


FIG. 3.—Effect on spore production of exposing *Fusarium cepae* cultures at 60 cm. from quartz mercury arc through vitaglass for various periods of time on six successive days.

fungi had been allowed to grow for one day, the unirradiated plates were exposed for the same periods (15, 30, and 60 minutes) to the mercury arc on three successive days. At the end of this time all plates were examined. Those with the medium irradiated before in-

TABLE I

COMPARATIVE EFFECT OF ULTRA-VIOLET RADIATION AND OF TEMPERATURE ON GROWTH AND SPORULATION OF MACROSPORIUM TOMATO AND FUSARIUM CEPAE

| TREATMENT  | TEMPERATURE IN °C. DURING EXPOSURE |      |      |      |      |      | RESULTS OBTAINED 5/30         |                        |                            |                               |
|--|------------------------------------|------|------|------|------|------|-------------------------------|------------------------|----------------------------|-------------------------------|
|  | 5/24                               | 5/25 | 5/26 | 5/27 | 5/28 | 5/29 | Fusarium                      |                        | Macrosporium               |                               |
|  |                                    |      |      |      |      |      | Average daily growth in (mm.) | Macrospores (per cent) | Average daily growth (mm.) | Spores                        |
| Exposed under vitaglass covers 1 hour daily (transmission to 2650 A.u.).....     | 40.0                               | 32.5 | 28.0 | 29.0 | 30.5 | 30.0 | 4.0                           | 50                     | 3.2                        | Abundant                      |
| Exposed under Jena petri dish lids 1 hour daily (transmission to 2800 A.u.)..... | 40.0                               | 32.5 | 28.0 | 29.0 | 30.5 | 30.0 | 4.5                           | 40                     | 3.3                        | Abundant                      |
| Exposed under ordinary glass covers 1 hour daily (transmission to 3020 A.u.)     | 40.0                               | 32.5 | 28.0 | 29.0 | 30.5 | 30.0 | 4.8                           | 10                     | 3.3                        | Many spores (less than above) |
| Petri dish covered with black paper, placed under arc 1 hour daily.....          | 35.5                               | 31.0 | 26.0 | 24.0 | 27.0 | 26.0 | 5.5                           | 1                      | 3.3                        | Few                           |
| Petri dish placed in incubator 1 hour daily.....                                 | 40.0                               | 35.0 | 29.0 | 28.0 | 30.0 | 33.0 | 3.0                           | 3                      | 2.0                        | Rare                          |
| Petri dish kept in diffuse light of laboratory.....                              | 25.5                               | 25.0 | 23.0 | 20.0 | 20.0 | 22.0 | 5.0                           | 2                      | 3.3                        | Very few                      |

oculation showed no increase over control plates kept in diffuse light, but those exposed after inoculation all showed a great increase in spore production.

The work thus far showed that the rays produced by a quartz mercury arc are effective in inducing sporulation. Since many radiations in the visible spectrum above 4000 A.u. were also produced,



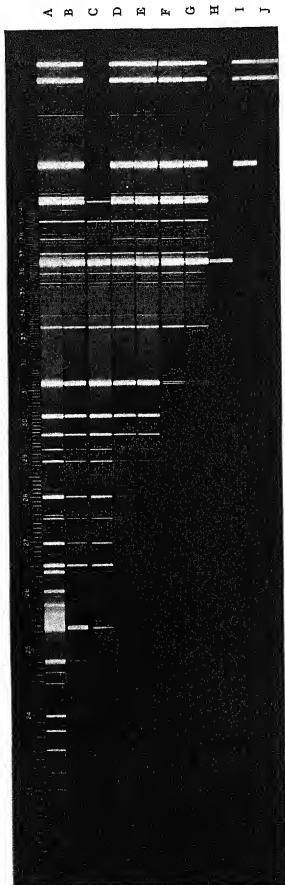


FIG. 4.—Spectrograms of quartz mercury arc direct and through various filters: A, direct arc (2230-6690 Å.); B, Corning Corex A (980 Å), (2300-6690 Å.); C, Correx Red Purple (986 Å), (2535-4340 Å.); D, vitaglass (2650-6690 Å.); E, petri dish (2800-6690 Å.); F, glass no. 1 (3020-6690 Å.); G, Corning (G586AW), (3334-3690 Å.); H, Noviol A (4340-6690 Å.); I, Noviol C (4880-6690 Å.). Some faint lines showing lowest limits of transmission are lost in reproduction.

it seemed desirable to try to determine the effective region by the use of filters. Consequently a number of filters, each limited in its transmission to definite regions of the spectrum, were procured with the idea of forming a series which would delimit the various portions of the spectrum. The spectrograms of these filters together with that of the mercury arc are shown in fig. 4.

Numerous experiments were then conducted in which these filters as well as the direct lamp were used to test the effectiveness of the

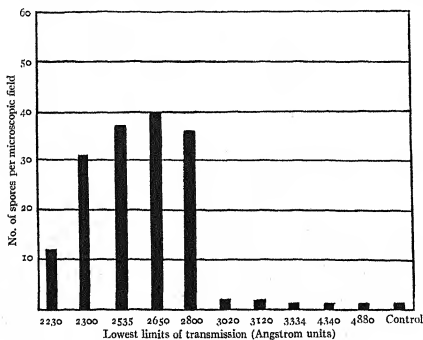


FIG. 5.—Composite graph showing effect on spore production of all experiments in which *Macrosporium tomato* was exposed through various filters at 40 cm. from the quartz mercury arc for 15 minutes.

various radiations upon sporulation of the fungi under consideration. The results of typical experiments with the tomato *Macrosporium* are shown in fig. 5. The most pronounced results were obtained by use of filters whose lower limits of transmission lie between 2535 and 2800 Ångstrom units. Optimum sporulation occurred with the filter whose lower limit was 2650 A.u. It will be noted that direct exposure to the arc caused a marked increase in spore production as compared with the controls, but less increase than that produced with any filter that eliminated the shortest rays but transmitted those between 2300 and 2800 A.u. The inhibiting effect of the short

rays from the direct arc is shown in the appressed, poorly developed mycelium (fig. 15 B), as well as in the amount of spores produced. There was practically no influence on the rate of growth when filters were used (fig. 16 A, B). The fungus on each of these plates grew at approximately the same rate as the controls, which showed an average daily increase of 3.5 mm. in diameter. The irradiated plates

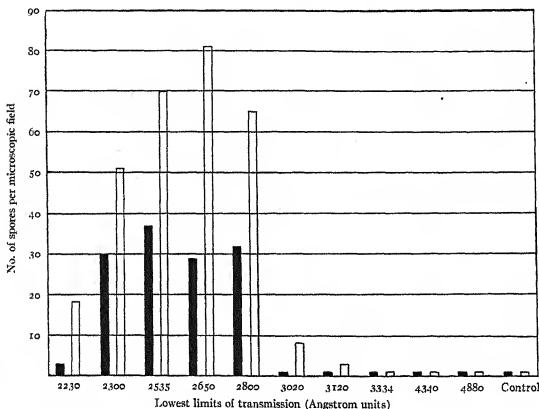


FIG. 6.—Effect on spore production by exposing cultures of *Macrosporium tomatum* to quartz mercury arc at 40 cm. through various filters for 15 minutes on two and three successive days: heavy line, 2-day exposure; light line, 3-day exposure.

showed a grayish green mycelial growth (fig. 15 A), whereas the controls were grayish brown and tended to be slightly more flocculent (fig. 15 C).

The effects of varying the number of exposures are shown in fig. 6. Plates exposed two successive days for 15-minute periods showed upon examination on the third day a very decided increase in spore production, varying from three times as many spores as the control in the case of exposure to the direct arc, to thirty-seven times as many with the use of the Correx Red Purple filter which transmits

down to 2535 A.u. Readings made after three daily exposures of 15 minutes each showed a great increase in all instances where filters which transmit between 2300 and 2800 A.u. were used. There was no change in the control. The maximum stimulation (eighty-one times as many spores as control) was obtained by use of the vitaglass filter which did not transmit below 2650 A.u. In all experiments there was a very sharp decline in the number of spores produced with filters which transmit nothing below 3020 A.u. There was a slight increase in spore production when cultures were irradiated through filters which transmit down to 3120 A.u., but, as shown in the graph, no filters whose lower limits of transmission are above this line of the spectrum were effective in inducing spore production in this species of *Macrosporium*.

Fig. 7 shows the results of exposing *F. cepae* through the various filters, for 15 minutes each on one, two, and three days. This graph is an average of four experiments. The highest percentage of macrospores was obtained by using the vitaglass filter which transmits to 2650 A.u. An increase in the number of exposures increased the percentage of macrospores. The percentage of macrospores produced under vitaglass in three days, as shown in this graph, is 8.3. In some other experiments, however, as high as 17.5 per cent macrospores was produced in three exposures of 15 minutes each (fig. 10). In all experiments, however, the numbers of macrospores produced under these filters bore the same relation to each other as that indicated in fig. 7, with the peak at 2650 A.u. In experiments with exposures of an hour the results followed the same curve.

No increase in production of macrospores occurred with filters transmitting no lower than 3334 A.u. The open arc and the two filters transmitting below 2650 A.u. caused stimulation but less than that produced by exposure through vitaglass. That this decrease in spore production is due to the presence of the shorter lethal rays in these three cases is upheld by microscopic examination. Many of the spores were plasmolyzed, and some, especially those exposed to the open arc, were disrupted. The contents of the plasmolyzed cells of conidia and mycelium were broken up into small globular masses. SIBILLA (9) reports a similar condition for *F. fuliginosporum* and *F. echinosporum*. In the case of disrupted spores these globules were

free and showed as loose aggregations among the hyphae. A marked decrease in growth rate and stunting of the colony (fig. 13 B) ac-

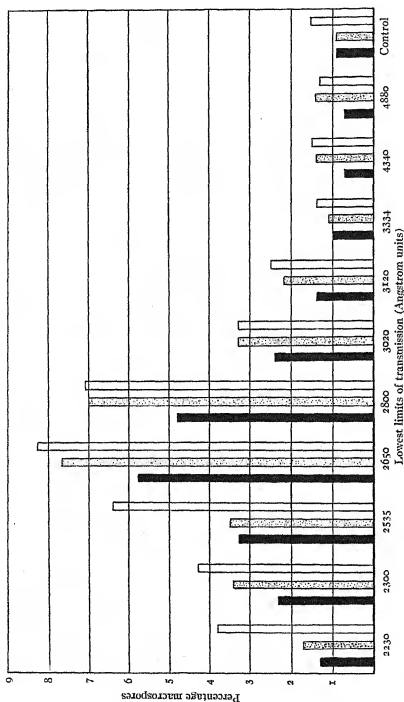


FIG. 7.—Effect on spore production by exposing cultures of *Fusarium cepae* to quartz mercury arc at 40 cm. through various filters for 15 minutes on one, two, and three days: heavy lines, 1 day 15 min.; dotted lines, 2 days 15 min. each; light lines, 3 days 15 min. each.

companied the lethal effect on the spores after exposure to the direct arc. The fungus was not entirely killed by three successive exposures of one hour, however, for it continued to grow and produce

spores. Of the spores present 24 hours after the third exposure, 18 per cent germinated in three days in distilled water.

The three filters whose lower limits of transmission lie between 2800 and 3120 A.u. also showed an increase in the percentage of macrospores over the control, the percentage varying inversely with the lower limit of transmission. The smaller increase in percentage of macrospores obtained when these filters were used as compared with that occurring with the use of vitaglass is not, however, due to lethal effects. Microscopic examination of spores and mycelium produced under these conditions revealed only normal cells (fig. 1D). Irradiation through these filters produced no consistent colony stunting, but the growth rate was approximately the same as in the control. Since there was no microscopic evidence of lethal effects, the slight decrease in growth rate (fig. 14) on exposure through these filters and through vitaglass might seem to be the result of the metabolic changes accompanying the assumption of sporulation rather than of direct inhibition caused by irradiation. In the case of *Macrosporium* no decrease in growth rate occurred with filters which transmitted no lower than 2800 A.u. (table I), and the decrease with vitaglass was so slight as to be negligible (fig. 16), although marked increase in sporulation occurred. In neither fungus was inhibition in growth rate, due to increased temperature, accompanied by sporulation (table I).

Since by the use of the vitaglass filter a maximum number of macrospores was obtained, this filter was used in experiments to determine the minimum exposure necessary for inducing an increase in the number of macrospores. Fig. 8 shows the results of exposures for various lengths of time, from one-hundredth of a second to one hour. A photographic shutter was used in making all exposures shorter than one minute. As shown in fig. 8, there was no definite increase in the percentage of macrospores produced with exposures less than 30 seconds on three successive days, although an exposure of one second gave a marked increase in the total number of microspores and in the percentage of septate microspores. The percentage of macrospores produced varied directly with the length and number of exposures. (In the particular experiment shown in this graph there was a slight irregularity in the 15-minute exposure for one day.)

It will be noted, however, that three successive short exposures are more effective than a single exposure of a duration equal to, or even

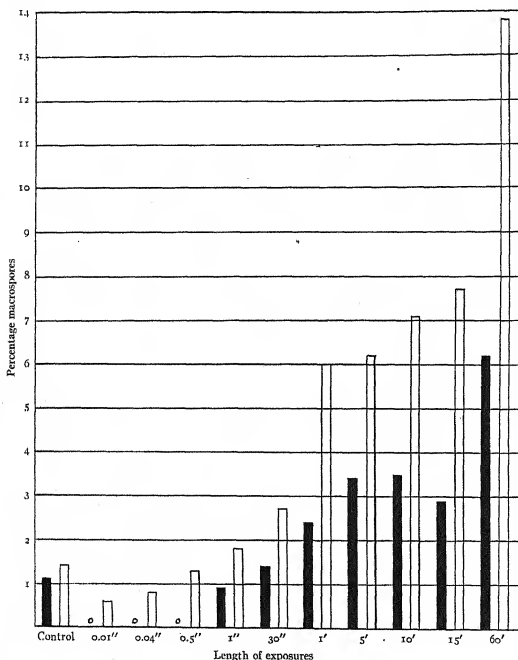


FIG. 8.—Effect on spore production by exposing cultures of *Fusarium cepae* to quartz mercury arc at 40 cm. through vitaglass for various periods of time on one day and for three successive days: heavy lines, 1-day exposure; light lines, 3-day exposure.

somewhat greater than their total. For example, a 1-minute exposure on each of three days induced the production of more spores

than five minutes on one day; and ten minutes on each of three days was more effective than one hour on one day. In a similar experiment with the filter transmitting down to 2300 A.u., a single exposure of 30 seconds was sufficient to produce an increase in percentage of macrospores.

In experiments with *Macrosporium* in which a Corex A filter transmitting down to 2300 A.u. was used, a slight increase in spore production was evident with a single exposure of one-tenth of a second. With the use of vitaglass, three successive daily exposures of one second produced a slight increase, while with a 30-second exposure a decided increase occurred. The more favorable results obtained with *Macrosporium* on exposures to the shorter wave lengths, as compared with *Fusarium*, is in accordance with the work of others, who found that pigmentation was a protection against the lethal action of these rays. Microscopic examination of cultures exposed for longer periods of time to the direct arc and to the rays transmitted by the Corex A (2300 A.u.) and Corex Red Purple (2535 A.u.) filters showed no disrupting of spores such as appeared in *Fusarium* under similar exposures.

Since increased spore production occurred with all the filters transmitting as low as 3120 A.u., but the amount of increase varied with the different filters, the question arose as to whether the results obtained were due to differences in the wave length or to intensity. As a preliminary attempt to determine what part intensity played in the stimulation of spore production, petri dishes with vitaglass lids were inoculated with *Macrosporium* and exposed for fifteen minutes, at varying distances from the arc (10, 20, 40, 60, 80, and 100 cm. respectively). Readings were made after one exposure and again two days later after three exposures. The results, as recorded in fig. 9, show that the maximum number of spores occurred in the plates exposed at a distance 20 cm. from the arc. The plates exposed at 40 and 60 cm. were practically the same in spore production, but a noticeable decrease in the number of spores was evident in the plates at 80 cm. and 100 cm. The cultures irradiated at 10 cm. gave about the same results as the two more distant ones. Perhaps at this close proximity to the arc there was some inhibitive effect due to the short waves transmitted by the vitaglass. The intensity at 80 cm., where



the big drop in spore production occurred, would be one-sixteenth (four-sixty-fourths), of that at 20 cm., where maximum production occurred. With this decrease in intensity is correlated a decrease to approximately one-third the number of spores, while in the case of the 40 and 60 cm. plates, intensity one-fourth and one-ninth as great, respectively, is correlated in both cases with a spore production two-thirds as great as the 20 cm. plates. Intensity is evidently a factor in the limitation of the number of spores produced.

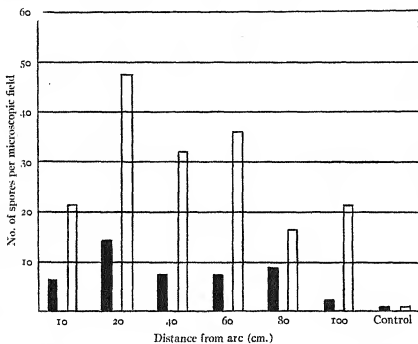


FIG. 9.—Effect on spore production by exposing cultures of *Macropsorium tomato* to quartz mercury arc through vitaglass for 15 minutes at varying distances on one day and for three successive days: heavy lines, 1-day exposure; light lines, 3-day exposure.

Cultures of *Fusarium* exposed at 10, 20, 40, and 60 cm. from the arc gave practically identical results. The 3-day exposures of fifteen minutes each at 40 cm. showed a slightly greater increase in percentage of macrospores (fig. 10); however, 1-hour exposures on three successive days at 40 and 60 cm. slightly reversed the results, so that it would seem that this slight variation is negligible. As in the case of *Macropsorium*, there was a definite decrease in the percentage of spores produced, when cultures were exposed at 80 and 100 cm., these cultures producing less than half as many spores as those exposed nearer the arc. Cutting down the intensity to one-thirty-sixth

was not enough to decrease the production of spores, but reducing it to one-sixty-fourth resulted in a decrease of approximately 50 per cent.

Since there is no adequate method of measuring the relative intensity of ultra-violet radiation passing through the various filters, an attempt was made to answer in part the question as to whether intensity or wave length was responsible for the variation in spore production under the various filters, by making longer exposures in

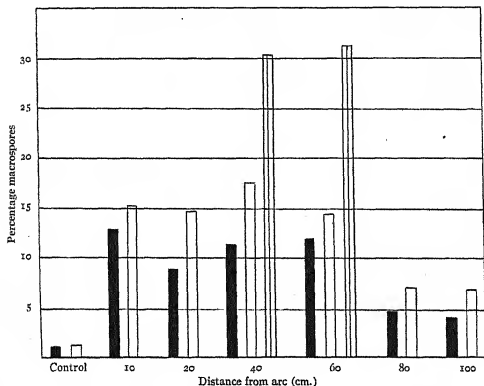


FIG. 10.—Effects on spore production by exposing cultures of *Fusarium cepae* to quartz mercury arc through vitaglass at various distances on one day for 15 minutes and on three days for 15 minutes and 1-hour periods: heavy lines, 1 day 15 min.; light line, 3 days 15 min. each; perpendicular line, 3 days 1 hour each.

the case of those filters which gave poor results with short exposure. Consequently, a series of exposures were made (fig. 11), the longest irradiation being six hours on each of three successive days. Exposures through Corning filter 586 A.W. (transmitting to 3334 A.u.) totaling eighteen hours did not induce sporulation, but the longer exposure did produce better results with the glass filters transmitting to 3020 and 3120 A.u. respectively. It will be noted, however, that

multiplying the exposure under these glass filters by 360 only doubled the spore production of a 1-minute vitaglass exposure. A 15-minute exposure under vitaglass produced twice as high a percentage of macrospores as was present under either of the other glasses following six hours' exposure, and even three successive daily exposures totaling eighteen hours, or seventy-two times as long an exposure, failed to produce as many spores as the vitaglass culture. In the spectrogram (fig. 4 *F*) the band at 3120 is prominent in the

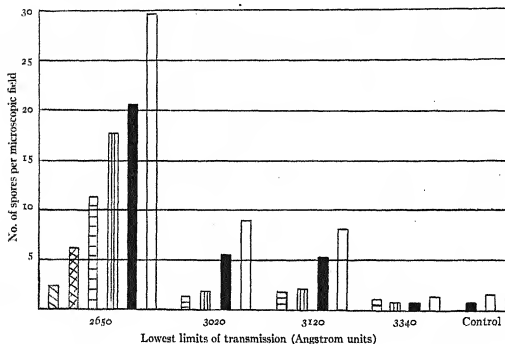


FIG. 11.—Effect on spore production by exposing cultures of *Fusarium cepae* to quartz mercury arc at 40 cm. through different filters for varying time periods: diagonal lines, 1 day 1 min.; cross lines, 3 days 1 min.; horizontal lines, 1 day 15 min.; perpendicular lines, 3 days 15 min.; heavy line, 1 day 6 hours; light line, 3 days 6 hours.

filter transmitting to 3020 A.u., and certainly does not appear photographically in the vitaglass spectrogram (fig. 4 *D*) to be seventy-two times as intense.

It would seem then, that if the difference in intensity between these two filters is primarily responsible for the difference in amount of increase in sporulation, it is the total energy over the whole region of the spectrum transmitted by vitaglass that is responsible and not the energy of any one band.

In the case of long exposures of *Macrosporium*, somewhat better

results were obtained with the glass filters (fig. 12), but even here multiplying the exposure under filter no. 6 by 360 only increased spore production by five and one-third times that of the 1-minute vitaglass culture. An average of eight spores was obtained with an irradiation of fifteen minutes through vitaglass, while an irradiation

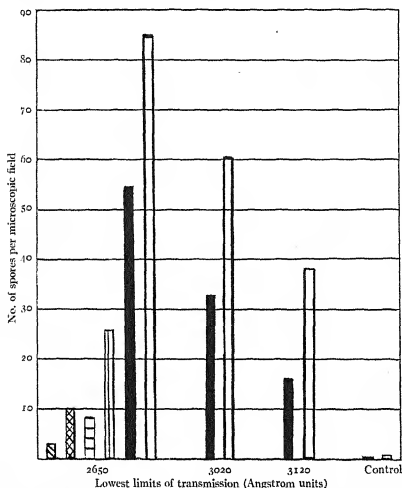


FIG. 12.—Effect on spore production obtained by exposing cultures of *Macropsorium tomato* to quartz mercury arc at 40 cm. through different filters for varying periods of time: diagonal lines, 1 day 1 min.; cross lines, 3 days 1 min. each; horizontal lines, 1 day 15 min.; perpendicular lines, 3 days 15 min. each; heavy line, 1 day 6 hours; light line, 3 days 6 hours each.

twenty-four times as long gave four times as many spores when filter no. 1 was used and twice as many spores with filter no. 6. Another experiment was conducted with *Macropsorium* in which the plates were exposed out of doors to bright sunlight, from about 1:00 to 3:00 P.M. daily for six days (May 3-9), the total exposure being

thirteen hours, fifteen minutes. The shortest detectable lines of the solar spectrum in Chicago at that time of the year are in the region of 3000 A.u., getting as low as 2990 on bright days (BUNDESEN 3). Filter no. 1 (lower limit 3020 A.u.) would therefore be transmitting almost as low as the vitaglass, since the shorter waves transmitted by vitaglass under the arc were not present in the sunlight. Exposures made under vitaglass, thin Jena petri dish (transmitting to 2800 A.u. under the arc), and filter no. 1, all resulted in plentiful sporulation with no detectable difference in quantity. In the control plates held in diffuse light spores were very rare. In a similar experiment with a series of *Fusarium* plates exposed to the sunlight for a total of nine hours on five successive days, more differentiation occurred between exposure under vitaglass and under filter no. 1. At the end of the period of exposure the vitaglass plates showed an exposed ochraceous salmon pionnotes 2.5 cm. in diameter. Macrospores averaged 40 per cent. The plates covered with filter no. 1 showed only a very little pionnotes in the very center, and the macrospores averaged 10 per cent. Control plates in diffuse light showed no pionnotes and only 3 per cent macrospores.

These experiments tend to show that the difference in amount of sporulation under the vitaglass and under glasses transmitting as low as 3020-3120 A.u. is not due solely to a difference in energy of the region from 3020 to 3120 A.u., but the total energy transmitted must be taken into consideration. Intensity is an important factor, for in experiments conducted after the manner of STEVENS (10) it was found that a 4-hour exposure through a spectrograph was insufficient to induce sporulation even under the most intense bands.

Further proof of the effectiveness of ultra-violet radiation in stimulating spore production is found in the following work. During the course of many experiments with *Macrosporium* the controls had been held for several weeks to observe whether sporulation took place as the cultures aged. In all instances it was found that very few spores were formed so long as the cultures were held in the ordinary diffuse light of the laboratory. Likewise cultures that had been irradiated through filters which excluded the rays effective in inducing sporulation have been found to show very few spores after being held for two or three weeks. Many of these plate cultures have been

subjected to irradiation through vitaglass, after they had failed to produce an appreciable number of spores within two weeks. In this manner it has been found that old cultures may be induced to form spores in abundance by irradiation with ultra-violet rays, while companion cultures in diffuse light remained unchanged. Good results have been obtained with 15-minute exposures for three successive days. The spores formed are found throughout the mycelium in the older part of the culture as well as in the younger hyphae along the edges of the plates.

Similar experiments with *Fusarium* were successful in increasing the percentage of macrospores. One exposure of fifteen minutes under vitaglass was sufficient to raise the percentage of macrospores on a plate which had previously been exposed for fifteen minutes on three successive days under filter 586AW (transmitting to 3334 A.u.), from less than 1 to 4.5 per cent. Former control plates exposed at the same time for fifteen minutes under vitaglass increased from 1.2 macrospores to 6.5 per cent macrospores, and plates previously exposed for three days for fifteen minutes to the full arc increased from 2.7 to 8.3 per cent macrospores. In the latter case the vitaglass, while still transmitting the stimulating rays, eliminated most of the lethal rays produced by the direct arc.

### Summary and conclusions

1. There is a definite stimulation of spore production in cultures of *Macrosporium tomato* and *Fusarium cepae* on exposure to ultra-violet radiation produced by a quartz mercury arc. Increased spore production appears to be a result of stimulation rather than an indirect result of inhibitory action.
2. Greatest spore production occurs when filters are used whose lower limits of transmission are between 2535 and 2800 Ångstrom units.
3. Slight stimulation results when filters transmitting no lower than 3120 A.u. are used, but those whose lower limits are 3334 A.u. or above are not effective.
4. Stimulation of spore production occurs with radiations of 2535 A.u. and shorter, but with these exposures there is also some lethal effect and inhibition of mycelial development. More killing occurs

with direct exposure to the mercury arc than with exposures through the filters. *Macrosporium* is more resistant than *Fusarium*.

5. Great increase in spore production occurs with radiations of 15-30 minutes. An irradiation of 36 seconds is usually sufficient to induce some increase.

6. Increasing the number of exposures is more effective than increasing the length of exposure. Three 15-minute exposures at 1-day intervals induces more sporulation than one exposure of 45 minutes.

7. Stimulation of spore production is not due to change in the culture medium as a result of irradiation.

8. The increased sporulation is not due to temperature.

9. Control cultures two weeks old, showing no spores, produced hundreds of spores on irradiation.

10. Long exposure to direct sunlight in May through filters transmitting no lower than 3120 A.u. induces abundant sporulation in both *Macrosporium* and *Fusarium*. This shows that if the intensity is great enough, wave lengths shorter than 3120 A.u. are not necessary for stimulation. The experiments with the quartz mercury arc, however, tend to show that the difference in amount of sporulation under vitaglass and under filters transmitting as low as 3020-3120 A.u. is not due solely to a difference in energy of the region from 3120 to 3020 A.u., but to a difference in the total energy transmitted.

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#### LITERATURE CITED

1. BOVIE, W. T., The action of light on protoplasm. Amer. Jour. Trop. Diseases Prev. Med. 2:506-517. 1915.
2. ———, The action of Schumann rays on living organisms. BOT. GAZ. 61:1-29. 1916.
3. BUNDESEN, H. N., LEMON, H. B., FALK, I. S., and COADE, E. N., Ultra-violet radiation from sunlight and incandescent lamps. Its transmission through window glass and substitutes. Jour. Amer. Med. Assoc. 89:187-189. 1927.
4. CHAVARRIA, A. P., and CLARK, JANET H., The reaction of pathogenic fungi to ultra-violet light and the rôle played by pigment in this reaction. Amer. Jour. Hyg. 4:639. 1924.

5. DUFRENOY, J., Action des radiations ultraviolettes sur les zoospores de *Blepharospora cambivora* Petri et de *Phytophthora omnivora parasitica*. Rev. Path. Veg. Entmol. Agric. 12:270-271. 1925.
6. FULTON, H. R., and COBLENTZ, W. W., The fungicidal action of ultra-violet radiation. Jour. Agric. Res. 38:159-168. 1929.
7. KUNKEL, L. O., A method of obtaining abundant sporulation in cultures of *Macrosporium solani* E. & M. Brooklyn Bot. Gard. Mem. 1:306-312. 1918.
8. Rands, R. D., The production of spores of *Alternaria solani* in pure culture. Phytopath. 7:316-317. 1917.
9. SIBILLA, CESARE, Azione dei raggi ultra-violetti e di alcuni anticrittogamici sui conidii di *Fusarium*. Anna. Ist. Superiore Agrario e Forestale. Florence. 2d S. Vol. I. (pp. 145 ff.) 1925.
10. STEVENS, F. L., The sexual stage of fungi induced by ultra-violet rays. Science N.S. 67:514-515. 1928.
11. ———, Effects of ultra-violet radiation on various fungi. Bot. Gaz. 86: 210-225. 1928.
12. TANNER, F. W., and RYDER, E., Action of ultra-violet light on yeast-like fungi. II. Bot. Gaz. 75:309-317. 1923.

#### EXPLANATION OF PLATE IV

Petri dish cultures of *Macrosporium tomato* and *Fusarium cepae* showing effects of ultra-violet irradiation:

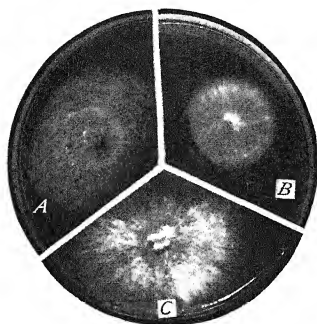
FIG. 13.—*Fusarium cepae*: A, exposed to mercury arc at 40 cm. through vitaglass for 15 minutes; B, exposed direct to mercury arc at 40 cm. for 15 minutes; C, control grown in diffuse light.

FIG. 14.—*Fusarium cepae*, exposed to mercury arc at 40 cm. for 15 minutes under vitaglass plate, one-half of which was covered with filter no. 6, transmitting to 3120 A.u.: A, vitaglass portion (note pionnotes and difference in growth rate); B, filter no. 6.

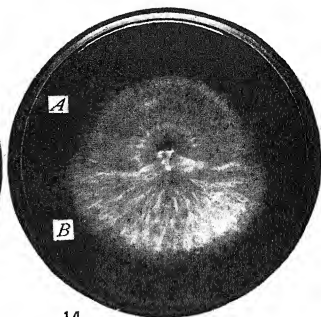
FIG. 15.—*Macrosporium tomato*: A, exposed to mercury arc at 40 cm. for 15 minutes through vitaglass; B, exposed direct to mercury arc at 40 cm. for 15 minutes; C, control grown in diffuse light.

FIG. 16.—*Macrosporium tomato* exposed to mercury arc at 40 cm. for 15 minutes under vitaglass plate, one-half of which was covered with filter no. 6: A, vitaglass portion (note darker color due to sporulation); B, filter no. 6 (note grayish color and slightly greater aerial growth).

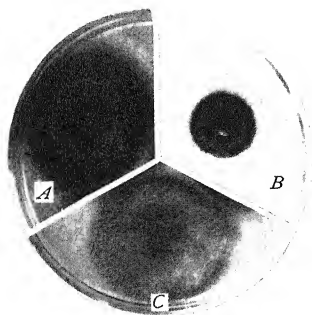




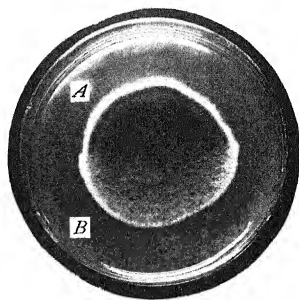
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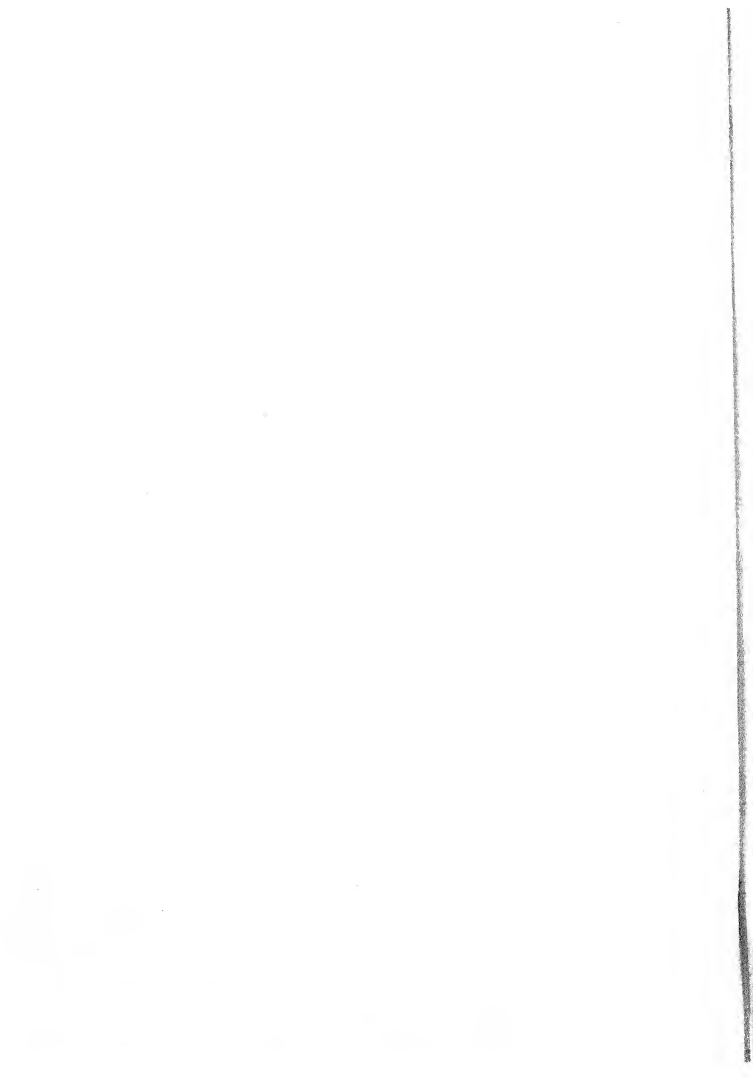


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16

RAMSEY and BAILEY on SPORULATION



## ORIGIN OF TISSUES OF SCHIZAEA PUSILLA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 400

D. R. BARTOO

(WITH TWENTY-SEVEN FIGURES)

*Schizaea pusilla* (Pursh), native of the pine barrens of New Jersey, is a stranded relic of a past age when tropical plants grew far to the north (MAXON 13). Although at present it is found only in two other regions (Nova Scotia and Newfoundland), there is little doubt that its ancestors flourished in early Mesozoic times. Its local distribution in widely separated regions has long been of interest to the plant geographer, but at present it is agreed that in postglacial times it had a continuous distribution from south to north along the Atlantic coastal plain. The geographic factors accounting for its present limited distribution have been presented by HARSHBERGER (11) and FERNALD (9).

Collections were made October 1, near Forked River, New Jersey, where the plants are found closely associated with those of a typical bog. Surrounded by mats of *Sphagnum*, intertwined with *Lycopodium carolinianum* and liverwort species, the plants are kept constantly moist. Although *Sphagnum* is most prominent, *Drosera*, *Sarracenia*, and *Vaccinium macrocarpon* comprise a considerable part of the association. The most complete description of *S. pusilla* and its habitat is that of BRITTON and TAYLOR (4).

### Description

The tiny, upright, perennial rhizome, 5-10 mm. long and 1-2 mm. in diameter, is covered with small, brownish hairs. The roots and leaves arise spirally (acropetally) about the apex from derivatives of segments cut from a tetrahedral apical cell. The rhizome seldom branches (fig. 1).

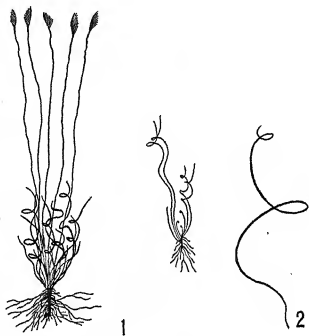
The linear, tortuous, sterile leaves form a rosette above the rhizome. They are slender and flattened, attaining a length of about 5 cm. The fertile leaves are also slender and flat, and rise to a height of 10-12 cm. above the sterile rosette. They are pinnate at

their extreme ends and each of the five to eight pairs of pinnae bears two rows of sporangia upon its abaxial side, one upon either side of the midrib. Both the sterile and fertile leaves exhibit the characteristic veneration of ferns (figs. 1, 2).

### Root

The root has a length of about 5 cm. and a diameter of 0.2 mm., all the tissues of which arise from segments cut from a tetrahedral apical cell (figs. 3, 4). The size of the root and the points of origin of the various tissues correspond to those of *S. rupestris* as observed by the writer (2).

The greatest axis of the apical cell lies in the main axis of the root, the three faces directed backward having the form of isosceles triangles. From the derivatives of the segments cut from these three faces the mature tissues of the root are ultimately formed. Segments cut from the forward equilateral face give

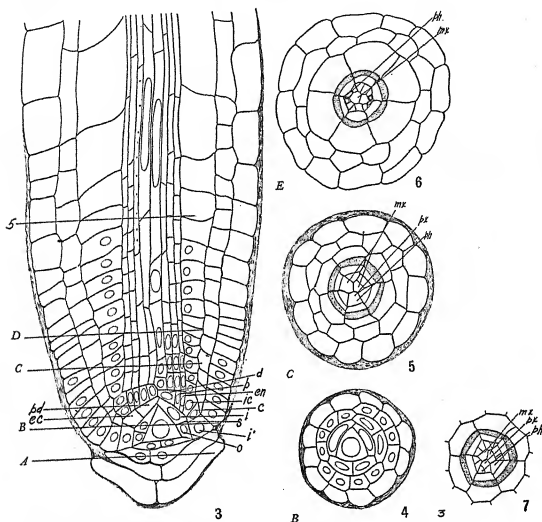


FIGS. 1, 2.—Fig. 1, habit sketch of mature plant, natural size; fig. 2, young plant showing rosette of tortuous sterile leaves, natural size.

rise to the root cap (fig. 3). It is obvious that the apical cell and root cap are pushed forward as the segments cut from the three equal faces divide and the resulting cells mature. It is to be noted that in reality segments are not "cut off," but with each nuclear division the protoplasmic mass is divided equally between the segment and the remaining apical cell (fig. 8).

Following the cell lineage from one of the three equal segments of the apical cell to the mature tissues of the root can best be accomplished by considering a median longitudinal section through an actively growing root tip (fig. 3). The immediate segment (s)

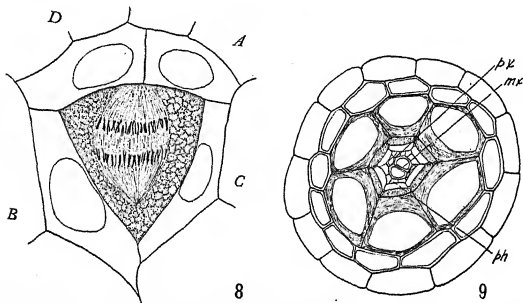
divides by a periclinal wall into an outer (*o*) and an inner cell (*i*). This outer initial cell (*o*) gives rise by one tangential division to the epidermis (*i'*), and to the outer layer of the cortex (*c*). In other



FIGS. 3-7.—Fig. 3, longitudinal median section of root tip: *o*, outer cell of immediate segment; *i*, inner cell; *i'*, initial cell of epidermis; *c*, initial cell of outer layer of cortex; *ic*, initial cell of inner layer of cortex; *en*, initial cell of endodermis; *p*, initial cell of pericycle; *d*, initial cell of desmogen strand; *ec*, mother cell to inner layer of cortex and endodermis; *pd*, mother cell to pericycle and desmogen strand. Fig. 4, transverse section through apical cell region of root tip as indicated by line *B* (fig. 3). Fig. 5, transverse section of root tip in region indicated by line *C* (fig. 3): *mx*, metaxylem; *px*, protoxylem; *ph*, phloem. Fig. 6, transverse section of root tip in region indicated by line *E* (fig. 3). Fig. 7, transverse section of root tip in region indicated by line *D* (fig. 3); stelar region still meristematic.

words, the outer cell of the immediate segment gives rise to the two outer tissue layers of the root. The inner cell, by two successive

periclinal divisions, gives rise to four cells, initiating the inner layer of the cortex (*ic*), the endodermis (*en*), the pericycle (*p*), and the desmogen (*d*). The inner layer of the cortex and the endodermis arise from a common mother cell (*ec*), as do also the pericycle and desmogen (*pd*). The innermost of the four cells derived from *i* varies in its method of division, depending upon its radial position and the tissue to which it is to give rise, as shown in transverse section.



FIGS. 8, 9.—Fig. 8, transverse section of apical cell undergoing cell division; cellular contents equally divided between segment and apical cell; A, B, C indicate order in which last segments were formed; fig. 9, transverse section of mature root: *px*, protoxylem; *mx*, metaxylem; *ph*, phloem.

Simultaneous with the periclinal divisions occurring (fig. 3), walls are formed in radial and tangential planes, as observed in transverse section (fig. 5). Soon after the division of the immediate segment giving rise to an outer and an inner cell, both cells divide radially, giving a sextet of inner cells somewhat tetrahedral in form surrounded by a sextet of isodiametric cells (fig. 26). The division walls of the three segments of *i*, however, passing from near the center of the outer face, do not meet at the common apex of the inner angles. All three walls diverge slightly, either to the right or left, so that the three alternate cells meet at the common center while the remaining alternate three do not extend to the center.

Each of the cells of the outer sextet undergoes two divisions in rapid succession (fig. 27), the first in a periclinal plane giving rise to *c* and *i*, and the second in a radial plane giving twelve cells per tissue layer for the epidermis and outer layer of the cortex (transverse section, fig. 6).

Two of the three alternate cells which meet at the center give rise to metaxylem (*mx*), while the third initiates the protoxylem and phloem (*px*, *ph*, fig. 5). The remaining three cells of the inner sextet (the three which do not occupy positions at the center) give rise to protoxylem and phloem (figs. 6, 7). This arrangement provides a diarch bundle consisting of from two to four protoxylem cells, two metaxylem cells, and from six to twelve phloem cells (fig. 9). Although metaxylem cells do not arise from segments directly opposite each other, they become part of a radially symmetrical bundle (fig. 9). The origins of the tissues of the root correspond to those of *S. rupestris*.

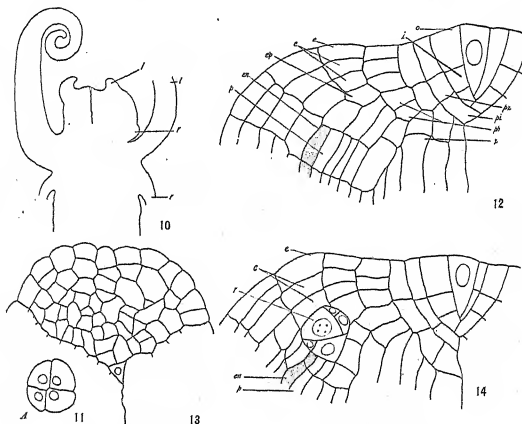
All the tissues of the root are differentiated very early. At a distance of 0.5 mm. from the apical cell all the tissues have been differentiated and cell division in all three planes has ceased. Cell division in a transverse plane ceases very early in xylem and phloem elements. There probably never occurs more than a single division after the xylem and phloem initials have been set apart, and it is very likely that many of these initial cells never undergo divisions in a transverse plane. The mechanics of elongation of the desmogen strand are quite evident. Many transverse divisions in the cortex, endodermis, and pericycle followed by growth of these cells in a longitudinal direction result in a stretching of the cells of the desmogen strand where transverse division has ceased or has never occurred (fig. 3).

Although protoxylem and metaxylem elements can be distinguished by differences in size and position at a distance of 0.2 mm. from the apical cell, it is at a distance of 1.8 mm. that protoxylem thickenings occur. Spiral thickenings occur in the metaxylem at a distance of 2.8 mm. from the apical cell. A definite determination between protophloem and metaphloem could not be made, but thickened walls occur in some of the phloem elements only 0.4 mm. from the apical cell, and it is probable that these represent proto-

phloem elements (fig. 3). No sieve areas were observed in the phloem.

### ROOT CAP

Segments cut from the forward face of the apical cell form the root cap (fig. 11). Two successive divisions take place at right angles to each other, so that four cells are formed from each segment.



FIGS. 10-14.—Fig. 10, median longitudinal section through rhizome, showing rudimentary leaves (*l*) and roots (*r*). Fig. 11, transverse section of root cap in region indicated by line *A* (fig. 3). Fig. 12, longitudinal median section through rhizome tip: *o*, outer derivative of immediate apical cell segment; *i*, inner derivative; *e*, epidermis; *c*, cortex; *ep*, mother cell of endodermis (*en*) and pericycle (*p*); *px*, mother cell of phloem and xylem; *pi*, pith. Fig. 13, transverse section through apical cell region of rhizome tip. Fig. 14, same as fig. 12, with root initial (*r*).

Often cells resulting from four or five of the original successive segments remain intact (fig. 3). The successive segments, giving rise to four cells each, usually form their walls in the same planes, but occasionally each alternate segment forms walls which are in planes  $45^\circ$  in a clockwise or counter-clockwise direction as viewed in cross-



section. The large cells from the older segments have scantier contents and are continually sloughed off as the root apex moves forward.

#### ROOT HAIRS

Root hair development begins at a distance of about 3 mm. from the root cap. The epidermal cells giving rise to root hairs (unlike those of *S. rupestris*) elongate in the direction of the axis of the root as the other epidermal cells do. The root hairs persist and were observed on old roots throughout their entire lengths.

#### RHIZOME

The tiny, brown rhizome is upright and attains a length of 6-12 mm. Just as in the root, all the tissues arise from a tetrahedral apical cell, derivatives from its three cutting faces giving rise to the various tissues of the rhizome. Numerous simple hairs arising from the superficial cells of the meristematic region serve as a protective covering for the growing tip. Roots and leaves, which have a spiral arrangement about the rhizome, arise acropetally from its apex.

Two of the three immediate segments cut from the apical cell may be observed in longitudinal section (fig. 12). Each of these divides first into an outer cell and then into an inner. The outer cell by two successive periclinal divisions gives rise to four cells, the outer three of which are initials to the epidermis and cortex. The innermost of these four cells (*ep*), by one periclinal division initiates the endodermis and pericycle. The inner cell of the original segment is divided by a periclinal wall into cells (*px*) and (*pi*), the former of which by one more periclinal division gives the phloem (*ph*) and xylem (*x*) initials. Derivatives of the latter (*pi*) become the pith. Simultaneous with the occurrence of the periclinal walls, anticlinal and radial walls occur, but no attempt was made to follow the sequence of cell lineage as seen in transverse section (fig. 13).

The transitional region of the stelar structure was observed in serial transverse sections. The transition from protostelic to siphonostelic type takes place as described by BOODLE (3), and the details will not be repeated here. The number of tracheids of the xylem increases in proportion to the number of leaves and roots. A rhizome having three leaves and two or three roots has sixteen to twenty

ty tracheids near its apex, the arrangement of which is still protostelic. By the time the fifth leaf trace has departed the aspects of a mature siphonostele are evident (fig. 25).

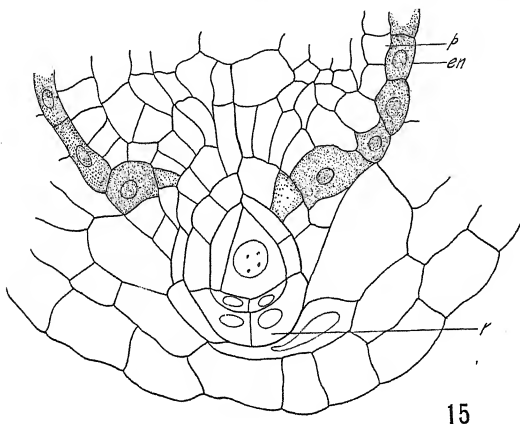
#### ORIGIN OF ROOTS FROM RHIZOME

Roots arise acropetally from the apex of the rhizome. They originate from a single cell which is a derivative of the immediate segment of the apical cell. The innermost of the four cells derived from the outer cell may become a root initial (fig. 14). In other words, the cell (*r*) whose sister cells (*ep*, fig. 12) divide to become endodermis and pericycle initiates the root. This root initial when situated only five or six segments from the apical cell of the rhizome becomes considerably larger than its neighboring homologous cells. The first segment is cut off either distal or proximal to the rhizome apex (fig. 14). The formation of this first wall is followed by a second so directed that it meets the first at an angle of  $120^{\circ}$ . A third wall meeting the first and the second at  $120^{\circ}$  completes the apical cell of the root. The fourth segment cut from this initial cell forms the root cap. Successive serial segments (either clockwise or counter-clockwise) are cut from the apical cell throughout the life of the root. The derivatives from three sets of immediate segments are differentiated into the various tissues of the mature root, as has already been described. Each tissue layer becomes continuous with the corresponding layer of the rhizome as it differentiates from a common meristematic mass (fig. 14): The apical cell is oriented in such a way that the growth of subsequent derivatives from its immediate segments forces it outward through the cortex of the rhizome. Three or four complete series of segments arise before the root finally breaks through the cortex a short distance from the rhizome apex. As soon as it emerges from the epidermis it turns abruptly downward.

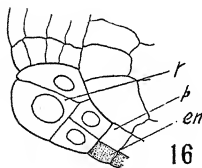
#### ORIGIN OF LEAF

The leaves are rather densely spiral as they arise acropetally from the apex of the rhizome. They apparently arise in equal numbers from each of the three sets of outer derivatives of immediate apical cell segments. After the first periclinal division an outer cell (fig. 18), which is set aside as a leaf initial, does not divide peri-

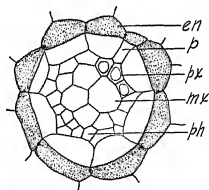
clinally as do the adjacent homologous segments (as seen in transverse section) which are to give rise to tissues of the rhizome. More-



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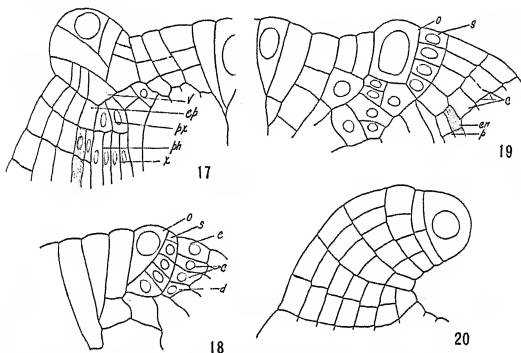
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FIGS. 15, 16, 21.—Fig. 15, transverse section through rhizome tip, showing rudimentary adventitious root in longitudinal section: *p*, pericycle; *en*, endodermis; *r*, root cap. Fig. 16, root initial (*r*) in transverse section of rhizome tip; two segments cut from initial root cell; *p*, pericycle; *en*, endodermis; fig. 21, cross-section of rudimentary leaf.

over, this leaf initial seldom divides by anticlinal walls as its neighbors do, but when three or four cells removed from the rhizome apex its first would-be "anticlinal wall" becomes oblique and cuts off an initial segment (figs. 18, 19). This first oblique wall usually occurs after the adjacent homologous segments have divided by anticlinal walls, and it is followed by the formation of a second wall so directed that it meets the first, forming a two-sided apical cell (fig. 17). In the meantime a series of periclinal walls has been occurring in the



FIGS. 17-20.—Fig. 17, rudimentary leaf arising from shoulder of rhizome tip; both leaf and rhizome in longitudinal section: *ep*, mother cell of endodermis and pericycle; *px*, cell showing division which originates phloem (*ph*) and xylem (*x*); *v*, inner cell of immediate segment giving rise to endodermis and stelar structures. Fig. 18, longitudinal section through rhizome tip showing leaf initial (*o*) with single segment (*s*) cut off. Fig. 19, same as fig. 18, showing origin of epidermis (*c*), endodermis (*en*), and pericycle (*p*). Fig. 20, longitudinal section through rudimentary leaf.

segments of the rhizome which are distal to the leaf initial. Two successive divisions arise in each segment (fig. 12), resulting in four cells the outer three of which become the epidermis and cortex, while the inner cell (*ep*) becomes the initial cell for the endodermis and pericycle. It should be noted that while these segments of the rhizome are undergoing division in a periclinal plane the leaf initial is cutting off oblique segments.

The two successive periclinal divisions of the rhizome seg-

ments are followed by two successive periclinal divisions of the first oblique segments cut off from the leaf initial (fig. 17), and the three outer cells which abut the three outer cells of the rhizome segments form the epidermis and the two cortical layers of the leaf. Thus the epidermis and cortical layers of the leaf and rhizome are continuous. The innermost of the four cell derivatives (*v*) of the immediate segment of the leaf initial corresponds in origin and position to the innermost of the four outer cell derivatives (*ep*, fig. 12) of the immediate segment of the apical cell of the rhizome, and, like it, gives rise to the endodermis and pericycle but in addition gives xylem, phloem, and pith (figs. 12, 17). The endodermis and pericycle, therefore, are probably cortical in origin in the rhizome but of stelar origin in the leaf.

It is obvious that both root and leaf cannot arise from the same immediate rhizome segment, since a leaf initial would include that portion (one-fourth) which becomes a root initial (figs. 14, 17).

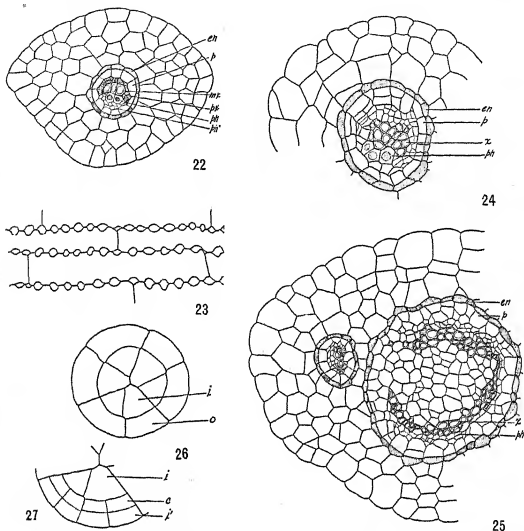
Successive segments cut off from the two-sided apical cell of the rudimentary leaf form a meristematic region which is at first in common with the meristematic region of the rhizome, so that differentiation into various tissues, even though of different origins, takes place simultaneously and the tissue systems of the leaf are from the beginning respectively continuous with those of the rhizome.

Only three or four pairs of segments are cut off from an apical cell of a rudimentary leaf. Each successive oblique wall becomes more nearly perpendicular to the main axis of the leaf, until a would-be oblique wall forms perpendicular to the axis, cutting off a hemispherical cell (fig. 20). It is while in this hemispherical cell stage that the cells of the leaf distal to the rhizome apex begin to grow more actively, causing the leaf to become "circinate" (fig. 20). After twelve or fifteen segments have been cut off from the hemispherical cell it divides into two equal quadrants. Subsequent cell division results in a marginal row of cells which finally lose their meristematic character.

#### TISSUES OF LEAF

The first tissues of the rudimentary leaf to differentiate are those of the epidermis and two layers of cortex (fig. 17). It has already been noted that the oblique segments cut from the apical cell divide

by two successive periclinal divisions into four cells. The outer three cells undergo no further division in a periclinal plane and become epidermis and cortex. The inner cell (*v*), by a periclinal



FIGS. 22-27.—Fig. 22, cross-section of rudimentary leaf, farther from apical than fig. 21: *ph*, phloem sieve tubes; *ph'*, protophloem. Fig. 23, longitudinal section through mature leaf. Fig. 24, transverse section through rhizome seedling showing protostelic arrangement: *en*, endodermis; *p*, pericycle; *x*, xylem; *ph*, pith cells. Fig. 25, transverse section through mature rhizome showing siphonostelic arrangement; leaf trace has departed from stele. Fig. 26, diagrammatic sketch of region near apical cell in transverse section: *i*, inner cell of immediate segment; *o*, outer cell. Fig. 27, same as fig. 26, showing subsequent divisions of outer cell.

division gives rise to cell *ep*, the common mother cell of the endodermis and pericycle, and to cell *px*, which initiates the phloem, and pith (fig. 17).

The endodermis, which consists of a single layer of ten to twelve cells as seen in cross-section, becomes very conspicuous when only 0.2 mm. removed from the leaf tip, because of its deep staining reactions to safranin. It is quite obvious that these endodermal cells and the adjacent pericycle cells originate from common mother cells (fig. 22).

Just as in the root, the protophloem is the first of the desmogen strand cells to assume thickened walls. First a single cell toward the abaxial side of the leaf becomes conspicuously blue (aniline stain) and somewhat thicker walled at a distance of about 0.25 mm. from the leaf tip (fig. 21). Serial sections show this conspicuous area gradually increasing to the adjacent cells and finally including (at about 1 mm. from the tip) all of the protoxylem cells (fig. 22).

Directly opposite the newly formed protophloem elements (toward the adaxial side) the protoxylem tracheids begin to assume their characteristic thickenings at a distance of 0.7 mm. from the tip (fig. 21). At first there is a single cell; then increasing to three, five, and nine, until at a distance of 1 mm. from the tip from sixteen to twenty protoxylem cells have been formed (fig. 22). It is at about this level that the three metaxylem tracheids become thick-walled. The two large metaphloem sieve tubes soon take on their characteristic blue walls (aniline blue).

It is quite evident, then, that the tissues of the desmogen strand become functional from point of time in the following order: protophloem, protoxylem, metaxylem, metaphloem. At a distance of 1.2 mm. from the tip all the tissues of the leaf are clearly differentiated (fig. 22). The arrangement of the bundle is collateral, as is usual in leaves. The whole consists characteristically of ten to twelve protophloem elements, fifteen to twenty metaxylem elements, three large metaxylem tracheids, and two large metaphloem sieve tubes.

#### Discussion

The present investigation is largely anatomical. It deals mainly with the origins of tissues and tissue organs. However, much valuable information has been obtained from descriptions and figures of the mature tissues of various species of *Schizaea*. PRANTL (14) describes the mature tissues of the root, rhizome, and leaf of *S.*

*penulla*, and shows the mode of origin of its sporangium. He "would like to consider" the endodermis as stelar in origin since it and the pericycle arise from common mother cells. He noted that the endodermis and pericycle are sister cells in the smallest strands of leaf veins. PRANTL's findings are not in agreement with the generally accepted histogenetic idea of HANSON that endodermis is cortical and pericycle stelar in origin. It is also interesting to note that more recent investigations of the origins of tissues in pteridophytes has revealed many exceptions to HANSON's theory.

BOODLE (3), in his monograph on the anatomy of the Schizaeaceae, deals chiefly with the structures of vascular tissues of the stem and petiole. He noted that there are only three layers of cells outside the endodermis in the root of *S. digitata*, and that the thickenings on the inner and radial walls of the inner cortical layer show distinct stratifications. Because the radial walls of the pericycle, endodermis, and inner layer of the cortex coincide he stated that the pericycle (as well as the endodermis) appears to be cortical.

BRITTON and TAYLOR (4) described the tissue arrangement of the transverse section of a mature root of *S. pusilla*, stating that "there is an endodermis of two layers and that the central cylinder is like that described by PRANTL for *S. penulla*." No doubt the pericyclic layer was mistaken for endodermis; however, in no case have BRITTON and TAYLOR recorded an attempt to trace the tissues to their origins.

TANSLEY and CHICK (15) found the cortex of the stem of *S. malaccana* to be five to eight cells thick. They state that these cortical cells are frequently packed with starch grains and that they always contain considerable mucilage. They noted that the radial walls of the endodermal cells are suberized "in the usual way," and that they are of the same size and correspond to their sister cells of the pericycle. They state that they "can hardly imagine that the sheath layers are not phylogenetically identical in monostelic ferns." In a figure of a longitudinal section through a stem tip (*S. digitata*) they show that the endodermis and pericycle are cortical in origin.

CONARD (8), in his investigation upon *Dicksonia*, found that the endodermis of the root is cortical in origin while the pericycle is



stelar. In the rhizome, however, he showed that both the external and internal endodermis are stelar.

CHANG (7), working upon the origin of tissues in the rhizome of *Pteris aquilina*, found that both pericycle and endodermis are stelar, while NAGELI and LEITGEB (1868) reported that the endodermis of the root of the same species is cortical. The recent work of JOHNSON (12) upon *Equisetum scirpoides*, and of BARCLAY (1) upon *Selaginella wildenowii*, confirms the findings of CHANG in regard to the origin of the endodermis in stems.

GOEBEL (10) states that in the Filicinaceae the leaf primordia proceed from one cell which is a segment of the apical cell. He finds that in those leptosporangiate ferns which have been carefully examined, the leaf primordia have at first a two-sided apical cell. PRANTL (14) shows that the first walls formed in a leaf initial of *Lygodium palmatum* are oblique, forming a two-sided apical cell, which soon becomes hemispherical. CONARD (8) states that the leaf initial in its earliest stages (*Dicksonia*) extends into the stem as far as the future boundary of medulla and plerome, but that no definite order could be discovered in the early divisions of the leaf initial. He reports that in its earliest stages the initial has four cutting faces, which are soon reduced to three and finally to two. In CONARD'S work upon the origin of adventitious roots from the rhizome of *Dicksonia* he found that the root initial arises "in that layer of cells which subsequently gives rise to both endodermis and pericycle." It should be observed that the location of the root initial of *Dicksonia*, as well as the behavior of its earliest cell divisions, forming a tetrahedral apical cell, corresponds to the initial stages of the root of the presently investigated species of *Schizaea*.

BOWER (5) presents a series of figures showing the transition stages from the protostelic to the siphonstelic condition in the rhizome of *Aneimia phyllitidis*. This series corresponds in general to that described by BOODLE (3) for *S. pusilla*, as already noted.

No findings have previously been recorded which show the origins of tissues of roots and rhizomes of the Schizaeaceae. The exact position of the leaf initial and its early subsequent divisions seem to have been undetermined in the Filicineae. The origin of

adventitious roots from a single cell, the sister cell of which gives rise to endodermis and pericycle, and its early subsequent divisions correspond to those of *Dicksonia* as described by CONARD.

### Summary

#### ROOT

1. All the tissues of the root arise from segments cut from a tetrahedral apical cell.

2. The tissues differentiate very early and in the following order: epidermis, cortex, endodermis, pericycle, protophloem, protoxylem, metaxylem, metaphloem.

3. The inner layer of the cortex and the endodermis originate from a common mother cell which is cortical (histogenetic according to HANSON).

4. The pericycle and desmogen strand originate from a common mother cell which is stelar.

5. The root cap formed from segments cut from the fore face of the apical cell remains very simple. Only four cells are formed from each immediate segment.

6. The root hairs persist throughout the life of the root.

7. The root has its origin in a single cell of the meristematic region of the rhizome, the sister cells of which give rise to endodermis and pericycle.

#### RHIZOME

8. All the tissues of the rhizome arise from segments cut from three faces of a tetrahedral apical cell.

9. The tissues differentiate early and in the same order as do those of the root.

10. The endodermis and pericycle have a common mother cell which is cortical.

#### LEAF

11. The leaf originates from the first outer derivative of the immediate segment cut from the apical cell of the rhizome.

12. The two-sided apical cell of a rudimentary leaf is transformed into a hemispherical apical cell and finally into a marginal row of meristematic cells.

13. The tissues differentiate in the same order as do those of the root. The endodermis and pericycle have a common mother cell which is cortical.

The writer expresses appreciation for the many valuable suggestions offered by Professor W. J. G. LAND.

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#### LITERATURE CITED

1. BARCLAY, B. D., Origin and development of tissues of stem of *Selaginella wildenowii* Bak. (unpublished).
2. BARTOO, D. R., Origin and development of tissues of root of *Schizaea rupestris*. BOT. GAZ. 87:322-331. 1929.
3. BOODLE, L. A., Further observations on *Schizaea*. Ann. Botany 17:511-536. 1903.
4. BRITTON, E. G., and TAYLOR, P., The life history of *Schizaea pusilla*. Bull. Torr. Bot. Club 28:1-6. 1901.
5. BOWER, F. O., The Filicales. vol. II. pp. 153-192.
6. CAMPBELL, D. H., Mosses and ferns. 3d ed. (pp. 384-390). 1918.
7. CHANG, C. Y., Origin and development of tissues in rhizome of *Pteris aquilina*. BOT. GAZ. 83:288-305. 1927.
8. CONARD, H. S., The structures and life-history of the hay-scented fern. Carnegie Inst. Washington. Publ. no. 90. 1908.
9. FERNALD, M. L., Botanical expedition to Newfoundland and Southern Labrador. Rhodora 14:109-162. 1911.
10. GOEBEL, K., Organography of plants. Vol. I. 1900.
11. HARSHBERGER, J. W., The vegetation of the New Jersey pine-barrens. 1916 (p. 5).
12. JOHNSON, M. A., Origin and development of tissues in the stem of *Equisetum scirpoides* (unpublished).
13. MAXON, WM. R., Ferns as a hobby. Nat. Geog. Mag. 47: May, 1925.
14. PRANTL, K., Untersuchungen zur Morphologie der Gefasskryptogamen. II Schizaeaceen. Lpz. 1881.
15. TANSLEY, A. G., and CHICK, E., On the structure of *Schizaea malaccana*. 493-510.

# SPIKELETS OF JOHNSON GRASS AND SUDAN GRASS

BETTY LONG

(WITH TWENTY-SIX FIGURES)

## Introduction

Johnson grass and Sudan grass are known to be near relatives, but very few of their differences, beyond the fact that the former is a perennial and the latter an annual, have ever been studied. Previous work<sup>1</sup> done on these grasses has been carried on mainly by experiment station workers who were interested in forage values, seedling experiments, chemical analyses, germination, and the eradication of Johnson grass where it had become a pest.

The spikelets of both grasses are arranged in groups, having one sessile and one pedicellate spikelet, or one sessile and two pedicellate in case of a terminal group (figs. 1-3). This grouping of spikelets in twos or threes, or even larger groups, is a more or less constant feature of all the Andropogoneae and Tripsaceae, and it also occurs in other tribes. The structure of these spikelets is very important in a basic study of the Andropogoneae. The flower parts (figs. 4, 5) of both grasses are essentially the same.

The present study has a twofold purpose: to find the morphological differences in the spikelets of the two grasses, and to describe the spikelets of these typical Andropogoneae from serial sections. No such descriptions have ever been published for any of the Andropogoneae, and this study may be used as a basis for future work in this tribe.

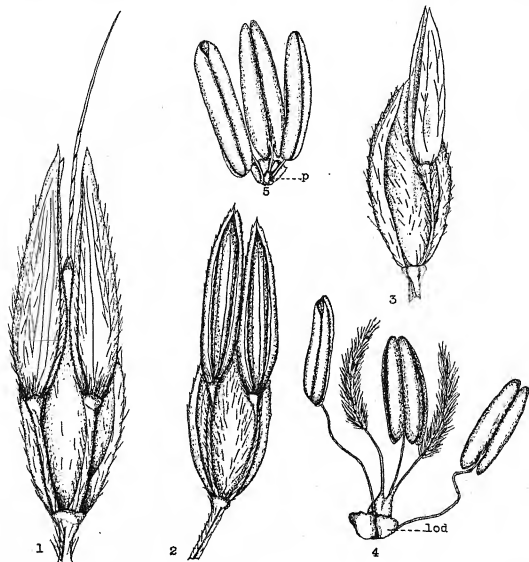
## History

Johnson grass has been given a number of names (1), among which are *Holcus halepensis* L. (1753), *Andropogon halepensis* (L.) Brotero (1804), and *Sorghum halepense* (L.) Persoon (1805). It has

<sup>1</sup> Since this was written, A. E. LONGLEY has presented a paper at the general section of the Botanical Society of America: Relationship between chromosome number and the annual and perennial habits of grass sorghums. He reports twenty chromosomes for the pollen mother cell of Johnson grass and ten for Sudan grass.

many common names (2), but Johnson grass is the one generally used in the United States.

The native country of Johnson grass is not definitely known. PIPER (9) states that it is "a native of the Mediterranean region



FIGS. 1-5.—Spikelets and flowers of Johnson and Sudan grass: fig. 1, terminal group of Sudan grass having one sessile and two pedicelled spikelets; fig. 2, same for Johnson grass; fig. 3, pair of spikelets of Johnson grass; fig. 4, flower of sessile spikelet of Johnson grass with abaxial lodicules (*lod*); fig. 5, staminate flower of pedicelled spikelet showing aborted pistil (*p*).

from the Madeira Islands to Asia Minor and southeastern Europe." The grass was first described from a specimen collected at the city of Aleppo, from which it received one of its common names and the

root of its specific name. The date of its introduction into this country was about 1830, under the name of *Holcus halepensis*. About 1840 it was cultivated extensively in Alabama.

*Andropogon sorghum sudanensis* Piper, *A. halepensis sudanensis* Piper, and *Holcus sorghum sudanensis* (Piper) Hitchcock are some of the names given Sudan grass. It is known as "garwari" in Sudan, but upon its introduction into this country was given the distinctive name of Sudan grass (10).

The exact nativity of Sudan grass is unknown, but it occurs in the same regions of the Old World as does Johnson grass, and was long thought to be a form of *Andropogon halepensis*, as Sudan botanists called Johnson grass (8).

The introduction of this grass came as a result of the search to find an annual to replace Johnson grass, which had become a pest because of its rhizomes. In 1909 Mr. R. HEWISON, director of agriculture at Khartum, Sudan, sent seeds, labeled *Andropogon halepensis*, common name "garawa," to PIPER (7).

According to BALL (1), "all cultivated sorghums are held to have been derived from the wild species *Andropogon halepensis*," or *Holcus halepensis* of LINNAEUS. OAKLEY (7), in speaking of Tunis and Sudan grasses, states:

They indicate the possible origin of the cultivated sorghums, resembling on the one hand Johnson grass in organization and vegetative characters, and to a similar degree the cultivated sorghums on the other. That they are primitive forms of sorghums can scarcely be doubted when their characters are carefully studied; and that they are more closely related to the sorghums than to Johnson grass is indicated by the fact that neither possesses underground rootstocks.

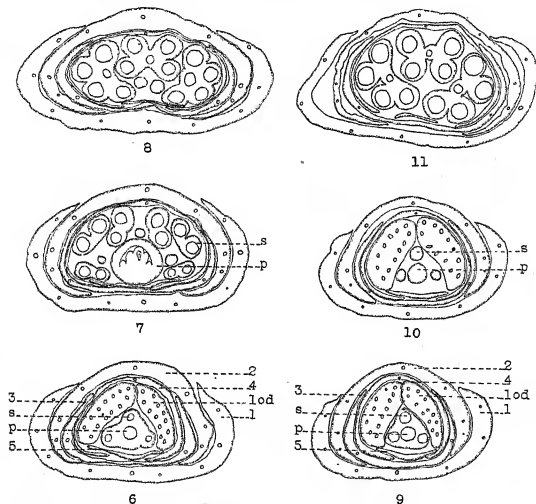
If Johnson grass, Sudan grass, and the cultivated sorghums are near relatives, they might be expected to hybridize. VINALL (10) and OAKLEY (7) say that Sudan grass and the cultivated sorghums hybridize readily. YOUNGBLOOD and CONNER (12) say that Sudan grass and Johnson grass cross. None of these investigators, however, has given any detailed descriptions of the resulting hybrid.

#### Material and methods

All the material for the study of these two grasses was grown on the University campus during the summers of 1926 and 1927. The Johnson grass was collected from plants grown from a single

rhizome, but the Sudan grass was collected from different plants grown from seeds, which were secured from a seed company.

The spikelets were imbedded in paraffin preparatory to sectioning. Better sections were obtained when the specimens were left only a short time in the higher alcohols, but for a longer period in chloroform and paraffin. Only young spikelets could be sectioned; the more mature ones, after pollination, were too hard.



FIGS. 6-11.—Transverse sections of sessile and pedicelled spikelets of Johnson grass at different levels: figs. 6-8, sessile spikelet; figs. 9-11, pedicelled spikelet; 1, 2, 3, 4, 5, bracts numbered from base of spikelet upward; lod, lodicule; p, pistil; s, stamen.

### Interpretation of data

#### JOHNSON GRASS

A cross-section of the sessile spikelet (figs. 6-8) shows that there are five bracts, two lodicules, three stamens, and a pistil. The num-

ber of vascular bundles in the two outer scales is variable and that of the next two bracts is constant; the fifth bract has no bundles in any of the specimens examined. Those of the lodicules are of a different nature from those of the bracts. The first two scales are very clearly the outer and inner glumes. There are four possible interpretations of the third bract, and consequently all the succeeding ones, depending on the number of flowers assumed to be present.

1. If only one flower is present the third bract might be the palea, the fourth the lemma, and the fifth a third lodicule. The two nerves in the third bract and the midrib of the fourth seem to indicate that such an interpretation is correct. This evidence is offset by the fact that bract 3 is on the outside of bract 4 and is attached at a lower level. This also requires the objectionable assumption that the whole floret is turned through an angle of  $180^\circ$ , for the lemma should be on the same side as the lower glume in a one-flowered spikelet. The fifth bract is very clearly not a third lodicule.

2. If only one flower is present the third bract might be the lemma, the fourth the palea, and the fifth a third lodicule. Evidence supporting this idea is that the third bract is on the outside of the fourth bract. Objections to this explanation are that the third bract does not have a midrib, the fourth bract has only one nerve and is not keeled; and the whole flower would still be turned through an angle of  $180^\circ$  as shown by the position of the stamens and lodicules.

3. If two flowers are present the third bract might be the palea of the lower flower, the lemma and flower being absent, the fourth bract the lemma of the second flower which is fertile, and the fifth its palea. Evidences favoring such a condition are the two-nerved third bract; the fourth bract having a midrib and being on the same side as the second glume; the two abaxial lodicules and single stamen on the side of the lemma; and the hyaline nature of the fifth bract. Embryonic remnants of the lost parts should appear below the third bract. The only objection to this interpretation is that the third bract should have its edges turned toward the first bract and not enfold all the higher structures. It is also hard to conceive a palea with no evidence of the lemma subtending the branch on which the palea is borne.

4. If two flowers are present, the third bract might be the lemma



of the first flower, the flower and palea being absent, the fourth the lemma and the fifth the palea of the upper flower. In addition to the evidence supporting this idea that the fourth and fifth bracts are those of a second flower is the fact that the third bract is turned toward the fifth bract and enfolds all the higher structures, which would be expected of a lemma in such a short spikelet. Rudiments of the first flower and palea should appear above the third bract. There is only one inconsistency, the fact that the third bract has no midrib.

The first problem was to find whether the spikelet is morphologically one- or two-flowered. Many spikelets were examined under the binocular microscope but none showed any conspicuous evidence of once having had another flower. On close inspection of the sectioned spikelets under the compound microscope all the specimens are found to have regions of embryonic tissue in a plane above the third bract and below the fifth, or between these in cross-section (fig. 22). This indicates that two flowers were once present, and that the third bract is the lemma of the lower flower.

Although this tissue points to the two-flowered condition, merely finding it does not prove that it is a remnant of the missing flower, but finding a vascular bundle to it and a midrib to the third bract would be more conclusive. In order to learn whether these bundles are present it was necessary to trace all of the vascular bundles in the spikelet.

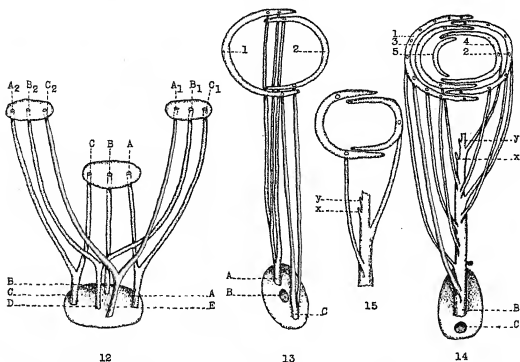
The origin of the bundles, and the levels of branching, have been worked out carefully but will not be described in detail because of their complexity. The main points can be seen from the diagrammatic figures.

Just below the place of attachment of the sessile spikelet on the rachis there are five vascular strands (fig. 23). The strands (fig. 12  $A_1$ ,  $B_1$ ,  $C_1$ ,  $A_2$ ,  $B_2$ ,  $C_2$ ) of the one or two pedicellate spikelets arise from these. Since those of the pedicellate spikelets branch independently, only those ( $A$ ,  $B$ ,  $C$ ) of the sessile spikelet will be considered.

From fig. 13 it will be seen that strands  $A$  and  $C$  supply only the extreme lateral edges of glumes 1 and 2. The bundles from  $A$  and  $C$  to glume 1 are branched at a lower level than those from  $B$

to the same glume; and the same condition exists with reference to glume 2. All other bundles of the spikelet have their origin in strand *B* (fig. 14). Two things will be noted: in both glumes the bundles to the right and left of the midrib originate below it; and the bundles to the first glume are attached below those of the second glume.

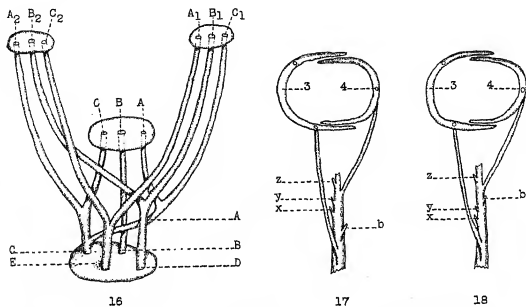
Below the midrib of bract 4 and on the opposite side of the axis is a rudimentary bundle (fig. 14*x*, fig. 24*x*), which is interpreted as



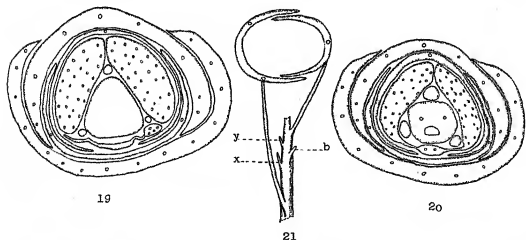
FIGS. 12-15.—Diagrams of vascular system of groups of spikelets of Johnson grass greatly elongated: fig. 12, primary bundles of rachis giving rise to two pedicelled and one sessile spikelets; fig. 13, main bundles supplying lateral edges of glumes only; fig. 14, main bundles supplying all other parts of spikelet; fig. 15, uppermost part of bundle (B) supplying pedicelled spikelet (fig. 13 is continuation of bundles A and C of fig. 12, fig. 14, continuation of bundle B of fig. 12); A, B, C, D, E, primary bundles in rachis; A, B, C, primary bundles in sessile spikelet; A<sub>1</sub>, B<sub>1</sub>, C<sub>1</sub>, A<sub>2</sub>, B<sub>2</sub>, C<sub>2</sub>, primary bundles of pedicelled spikelet corresponding to A, B, C of sessile spikelet; x, rudimentary midrib of bract 3; y, rudimentary bundle supplying lower flower once present and subtended by bract 3.

the midrib of the third bract. Here, as in the glumes, the bundles to the right and left of the midrib are below it and distinctly precede it in order of development; thus *x* is in a normal position in respect to other bundles of bract 3 according to the plan of the glumes, where the lateral bundles are below the midrib. This midrib, to-

gether with the lateral bundles, indicates that the third bract is a lemma, of either a lower or an upper flower.



FIGS. 16-18.—Diagrams of vascular system of spikelets of Sudan grass: fig. 16, primary bundles of rachis of Sudan grass corresponding to those of Johnson grass in fig. 12; fig. 17, upper part of bundle *B* in sessile spikelet; fig. 18, upper part of bundle *B* idealized; *b*, rudimentary bundle to left of midrib of bract 4; *z*, rudimentary bundle to third lodicule once present and still appearing occasionally.



FIGS. 19-21.—Cross-sections of sessile spikelet of Sudan grass and diagram of pedicelled spikelet: fig. 19, cross-section showing third lodicule well developed; fig. 20, same with fifth bract adnate to all three lodicules; fig. 21, upper part of bundle *B*.

A second rudiment (fig. 14y, fig. 24y) above the midrib of the fourth bract and on the opposite side may be explained as the bundle

to the lower flower when it was present. This indicates that the meristematic tissue represents a lower flower, and consequently that the third bract is a lemma subtending the lower flower.

According to the plan of the glumes, where all bundles to one scale are attached below any of the bundles to a higher bract,  $x$  is in a normal position; but contrary to it  $y$  of the first flower appears above those of the second floret. In all probability strand  $B$  at one time divided into two equal branches, one (now represented by  $y$ ) to the lower flower, and the other to the upper flower. During evolution, when the lower flower was lost, the rachilla became shortened, and the upper flower moved into a more upright position, so that this rudiment now appears to branch above the bundles of the fourth bract. Or it may be that in reality  $y$  branches below this bundle but because the plane of the section is oblique to the rachilla it appears above. The significant point is the order of attachment of bundles in the same bract, however, not the order of those of one bract with respect to those of another bract.

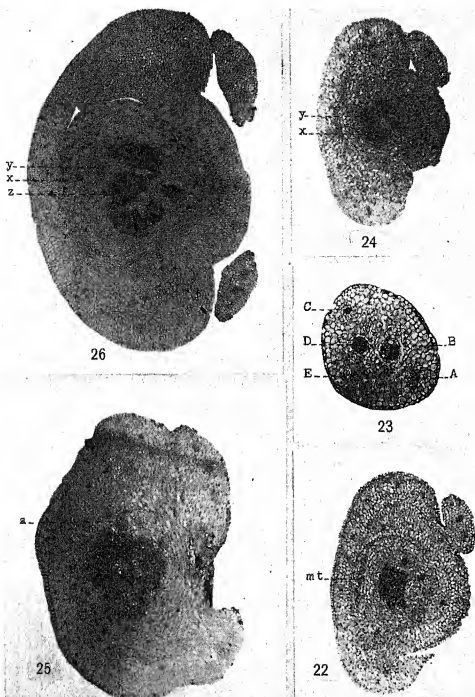
There are two primary bundles to each of the lodicules. In the pistil strand  $B$  divides into three parts, two of which enter the style, the third again dividing into four parts and supplying the posterior wall of the pistil and the ovule.

There is sufficient evidence to justify the statement that in the sessile spikelet of Johnson grass the third bract is the lemma of the first (lower) flower, which was lost long ago, and the fourth and fifth bracts the lemma and palea of the second flower which is fertile.

Having established the general plan of the vascular system and the parts of the sessile spikelet, and on the assumption that both spikelets were once perfect, observations were made on the pedicelled spikelet to see wherein these two differ.

The corresponding five bracts are present, offering the same possible interpretations as those of the sessile spikelet; and there are three stamens and two large lodicules (figs. 9-11). In addition, there is an aborted pistil, indicating that the pedicellate spikelet, which is now staminate, was once perfect. In all specimens examined meristematic tissue is present in the same position as in the sessile spikelet, again indicating a two-flowered condition.

In the pedicel there are three large strands (fig. 12  $A_1$ ,  $B_1$ ,  $C_1$ )



FIGS. 22-26\*.—Fig. 22, transverse section of sessile spikelet of Johnson grass showing meristematic tissue of first (lower) flower; fig. 23, transverse section below sessile spikelet of Johnson grass showing five primary bundles; fig. 24, transverse section of sessile spikelet of Johnson grass showing rudimentary midrib of third bract (x) and bundle to first flower (y); fig. 25, transverse section of sessile spikelet of Sudan grass showing rudiment of lateral bundle (a) of fourth bract; fig. 26, transverse section of sessile spikelet of Sudan grass showing rudimentary bundle (z) to third lodicule in addition to x and y of third bract and lower flower.

\* Photographs made by Professor WEATHERWAX. Abbreviations as follows: *mt*, meristematic tissue; *A*, *B*, *C*, *D*, *E*, primary bundles; *x*, rudimentary midrib; *y*, rudimentary bundle of lower flower; *a*, rudimentary lateral bundle; *z*, rudimentary bundle of third lodicule.

which correspond to the strands *A*, *B*, and *C* of the sessile spikelet, except for a few minor differences, so that it will not be necessary to describe the vascular system of the staminate spikelet. Both bundles *x* and *y* appear above the midrib of the fourth bract (fig. 15), but this can be explained by the reorganization of bundles, or the angle at which the section was cut. The fact that *x* is attached in the normal position higher than the lateral bundles to bract 3 is the significant evidence. There is no bundle to the aborted pistil of the upper flower.

From the study of these two spikelets it will be seen that Johnson grass at one time had a group of two perfect two-flowered spikelets, one of which was pedicellate; scales 1 and 2 are glumes, bract 3 is a sterile lemma, and 4 and 5 are the lemma and palea of the functional flower.

#### SUDAN GRASS

Cross-sections of the spikelets of Sudan grass show all parts corresponding to those of Johnson grass. Because of the similarity of the two species it seems plausible to interpret the third bract as the lemma of a flower that has been lost. Meristematic tissue is present in the same region as in Johnson grass.

In general both grasses have identical vascular systems; so that it will be necessary to mention only the variations which occur in Sudan grass. In the rachis below the sessile spikelet there are usually four vascular strands, one of which divides and the five resulting strands correspond to those of the first species (fig. 16).

At a level slightly above the branching of the lateral nerves of the third bract two rudimentary bundles (fig. 25*a*, fig. 17*b*) are observed and may be explained as the remnants of bundles to the left and right of the midrib of the fourth bract. These rudiments are in their normal position in respect to the midrib of the fourth bract, but not in respect to the bundles of bract 3.

Immediately above these and on the opposite side of the strand are two other rudimentary traces (fig. 17*x*, *y*). The lower one (*x*) is interpreted as the midrib of the third bract; the other (*y*) is explained as the bundles supplying the lower flower. Both of these bundles are in normal positions in respect to those of bract 3, but

neither in respect to those of bract 4. The ideal condition is shown in fig. 18.

The rudiment (fig. 17z, fig. 26) above the midrib of the fourth bract and on the opposite side is not present in Johnson grass, but, as will be seen later, it may be the bundle to a third lodicule and is in a normal position.

In Sudan grass there are three primary bundles to each of the two abaxial lodicules, while in Johnson grass there are only two.

Further inspection of twenty-five specimens shows that four have remnants of a third (adaxial) lodicule, which is in all cases adnate to the palea. In three of these the remnant is centrally located in the palea; in the fourth it is more fully developed and is adnate to one side (fig. 19). In all four cases there is a single bundle to this third lodicule. In three specimens this main branch remains as one or divides into two or three smaller branches; in the fourth the bundle divides further and forms many small bundles like those of the abaxial lodicules.

This third lodicule may have a bearing on the phylogenetic significance of the lodicules. Many theories concerning them are current. Miss FRANCIS (3) believes that the flowering scale (lemma) and the palea together constitute a three-parted calyx, the lemma being one part and the palea the other two parts grown together. It would probably follow that the lodicules represent a degenerate corolla. Since we now interpret the lemma as being attached to the rachis and subtending a short branch which bears the palea and the flower, her theory is not widely accepted.

HACKEL, in his earlier work at least (4), regarded the lodicules as equivalents of the palea. In discussing this subject in 1888 (5), he "has endeavored to prove that the anterior scales represent halves of a leaf which sometimes remains undivided, and can be regarded as a second, and the posterior scale as a third, palea." By anterior scales he means the two abaxial lodicules; the third which is sometimes present is adaxial. He explains that the difference in structure is due to physiological functions.

HITCHCOCK and many others apparently consider the palea as homologous with the prophyllum of vegetative parts of the plant. "The lodicules are interpreted by some to be homologous with the

divisions of a perianth of which only two divisions have usually persisted" (6).

If the third lodicule were explained according to HACKEL, it would be seen that the two abaxial lodicules forming the "second palea" in this case would have six primary vascular bundles; the "third palea," which is the third lodicule, would have one vascular bundle which should be attached above those of the second palea. In all cases the palea is adnate to both abaxial lodicules in low levels (fig. 20) and also to the third lodicule. It would be a very peculiar condition in which the first palea was attached at both sides to the divided second palea which was in a higher plane, and also to the third palea which was in a second higher plane. It is no more correct to interpret the abaxial lodicules as a palea because they are united in some grasses than it would be to say that in the case of Sudan grass the adaxial and one of the abaxial lodicules form a palea because they are united.

The other explanation seems more plausible. The palea may be adnate to the abaxial lodicules in low levels because of the crowded condition of the spikelet. It is not inconsistent that the adaxial lodicule, which is in the same plane with the abaxial ones, is attached to them and to the palea also. In those cases where the third lodicule is centrally located in the palea, these two structures seem never to have been differentiated at the growing point.

Cross-sections of the pedicellate spikelets show all the parts corresponding to those of the pedicelled spikelet of Johnson grass. All specimens have embryonic tissue above the third and below the fifth bracts. In contrast with the sessile spikelet of Sudan grass there is no evidence of a rudimentary lodicule.

The vascularization of this spikelet is essentially the same as that of the sessile spikelet below the third bract; above this there are a few differences (fig. 21). The rudiments  $x$  and  $y$  are in their normal positions in respect to bract 3; but those of the first floret are not normal in relation to those of the second floret.

### Summary

1. In both spikelets of both grasses the vascularization is essentially the same. The nerves to the right and left of the midrib in



every bract are attached below the midrib of that bract. All the bundles to one bract are attached below and on the opposite side of the rachis from any of those to the next higher bract.

2. Meristematic tissue of a first flower and a rudimentary bundle to it establish the fundamental two-flowered condition in both spikelets of both grasses. This is consistent with the now generally accepted opinion as to the nature of this spikelet. It is also consistent with the structure of the spikelet in the Paniceae, where both the lemma and palea of the lower floret are present, and a staminate or even a perfect lower flower is sometimes present; and in the Tripsaceae, where the same condition has been established.

3. The rudimentary midrib to the third bract and embryonic tissue above it establish the fact that the third bract is a lemma of a lower flower.

4. The aborted pistil suggests that the pedicelled spikelets of both grasses once had a perfect flower.

5. Some of the spikelets of Sudan grass have a partly developed adaxial lodicule; in the others this lodicule is represented by a rudimentary bundle.

6. Observations on the lodicules of Sudan grass seem to indicate that they are members of a perianth, all three of which are present in a few cases.

I wish to express appreciation for the CLARA JAVAN-GOODBODY Scholarship (1927-28) and a special fellowship (1928-29) which made this study possible; and for the valuable suggestions and encouragement given by Professor PAUL WEATHERWAX, under whose direction the work was done.

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#### LITERATURE CITED

1. BALL, C. R., The history and distribution of sorghum. *Bur. Pl. Ind. Bull.* 175. 1910.
2. ———, Johnson grass: report of investigations made during the season of 1901. *Bur. Pl. Ind. Bull.* 11. 1902.
3. FRANCIS, MARY E., The book of grasses. Garden City, N.Y. 1920.

4. HACKEL, ED., Untersuchungen über die Lodiculae der gräser. Bot. Jahrb. 1:335-361. 1881.
5. ———, The true grasses. Engl. Transl. New York. 1890.
6. HITCHCOCK, A. S., A textbook of grasses. New York. 1922.
7. OAKLEY, R. A., Some new grasses for the south. U.S. Dept. Agric. Year-book. 1912.
8. PIPER, C. V., Sudan grass, a new drought-resistant hay plant. Bur. Pl. Ind. Cir. 125. 1913.
9. ———, Cultivated grasses of secondary importance. U.S. Dept. Agric. Farmers' Bull. 1433. 1925.
10. VINALL, H. N., Sudan grass. U.S. Dept. Agric. Bull. 1126. 1921.
11. ———, Sudan grass as a forage crop. U.S. Dept. Agric. Farmers' Bull. 605. 1914.
12. YOUNGBLOOD, B., and CONNER, A. B., Sudan grass. Texas Agric. Exp. Sta. Bull. 172. 1915.

# EFFECT OF LOW TEMPERATURES ON GERMINATION OF IMPERMEABLE SEEDS<sup>1</sup>

W. F. BUSSE

## Introduction

The problem of hastening the germination of seeds having a dormancy due to an impermeable seedcoat has received considerable attention, since it is of both scientific and economic importance (2, 5). Among the various treatments that have been suggested, removing the seedcoat is one of the most direct and obvious methods that can be used. This may be done mechanically or by means of concentrated sulphuric acid, but both methods have very apparent disadvantages. Scratching or scarifying the seeds will cause many of them to become permeable, but with large irregular seeds like alfalfa this treatment injures many, and it also decreases their keeping power (4).

Recently DAVIES (3) found that subjecting clover and alfalfa seeds to pressures of 500–2000 atmospheres reduced the proportion of impermeable seeds. STAKER (10) and STEWART (12) found that dry heat reduced the impermeability of alfalfa seeds. The alternate freezing and thawing which seeds receive when they are planted in the late fall causes some to become permeable (8), but the freezing also kills the young plants and seeds which have imbibed much water. MIDGLEY (7) tested the effect of alternate freezing and thawing on hard seeds. He found the first freezing of moist alfalfa seeds to about  $-5^{\circ}$  C. caused some of them to become permeable, but subsequent freezings had little effect in reducing the impermeability. If the seeds were kept moist for several months, he found that as many seeds would become permeable without freezing as with freezing, and he makes the statement that the intensity of freezing had no effect on the increase in permeability.

In the course of some investigations of hard seeds carried out in

<sup>1</sup> Contribution from the Laboratory of Physical Chemistry of the University of Wisconsin.

this laboratory, the writer was led to study the effects of very intense freezing on seeds, down to the temperature of liquid air ( $-190^{\circ}\text{C.}$ ), with results which were rather surprising. It has long been known that dry seeds stand freezing in liquid air (I) and seeds having a high moisture content are killed by this treatment (IX), but very little work has been reported on the changes other than killing which are produced when the seeds are frozen at very low temperatures. It was found in this work that while freezing the dry impermeable seeds at moderately low temperatures had no effect on their germinability, freezing them at very low temperatures would enable the seeds to germinate promptly when placed under normal germinating conditions. In the case of some sweet clover seeds, freezing in solid carbon dioxide snow ( $-80^{\circ}\text{C.}$ ) had almost no effect on the impermeable seeds, but freezing in liquid air was very effective in destroying the impermeability without injuring the seeds. With alfalfa seeds, freezing to  $-20^{\circ}\text{C.}$  had little effect, but freezing to  $-80^{\circ}\text{C.}$  made nearly all of them permeable. These results were deemed of sufficient importance to justify a careful study of some of the factors involved, so the following experiments were performed.

### Experimentation

Several different lots of air-dry alfalfa, common sweet clover, and Grundy County sweet clover seeds, each of which contained a high percentage of impermeable seeds, were used in these experiments. The seeds were of the 1927 crop, and most of the tests were made during the winter of 1927-28, with the aging tests running through the following year. The alfalfa was machine hulled, but the clover seeds were hulled and cleaned by hand.

To treat the seeds, they were usually put in a small test-tube and this was then immersed in the refrigerant, which was contained in a Dewar flask. The seeds were then either given a rapid, non-uniform warming by pouring them from the test-tube onto a warm metal plate (treatment I), or else they were allowed to warm up very slowly by leaving them inside the Dewar flask until the refrigerant had evaporated and the flask had warmed to room temperature (treatment II). In a few cases the seeds were cooled comparatively slowly by suspending them several centimeters above the surface of

the refrigerant in the Dewar flask for several hours, while in other cases the seeds were poured directly into the refrigerant. In one experiment seeds were left in liquid air continuously for about six months. The germination tests were usually made by putting 100 seed samples on filter papers in crystalizing dishes, and keeping them moist with distilled water. The treatments and tests were repeated several times to check the results. A few samples of treated seeds were grown in soil in the greenhouse and it was found that the plants were perfectly normal.

### 1. FREEZING IN LIQUID AIR

1. EFFECT ON DIFFERENT SEEDS.—Table I, showing the effect of freezing several lots of seeds in liquid air, is typical of the results obtained with this refrigerant. The seeds given treatment I were

TABLE I  
EFFECT OF FREEZING IN LIQUID AIR ON GERMINATION

| SEED                                   | PERCENTAGE GERMINATION |             |              |
|--|------------------------|-------------|--------------|
|  | Control                | Treatment I | Treatment II |
| Grundy County sweet clover, Lot 1..... | 14                     | 76          | 90           |
| Grundy County sweet clover, Lot 2..... | 47                     | 70          | 70           |
| Common sweet clover, Lot 1.....        | 64                     | 86          | 94           |
| Common sweet clover, Lot 2.....        | 58                     | 83          | .....        |
| Alfalfa, Dakota.....                   | 68                     | 100         | .....        |
| Alfalfa, Idaho.....                    | 52                     | 96          | 100          |

kept in the liquid air from three to five minutes and then rapidly warmed, while the seeds given treatment II were slowly warmed.

The increase in germination from 14 to about 90 per cent found with Grundy County sweet clover shows the effectiveness of this treatment. The somewhat lower germination of the seeds that received treatment I is probably due to the fact that they were not kept in the liquid air long enough for all the seeds to be completely cooled. If the seeds that did not grow were given another freezing in liquid air, from 20 to 100 per cent of them would become germinable.

The length of time the seeds are kept at the liquid air temperature has almost no effect on the germination, at least for times of

cooling up to six months. This was shown by putting some sweet clover seeds into a flask that was kept filled with liquid air, and withdrawing seeds for germination tests every few months. Table II shows the results obtained.

The seeds kept in liquid air for 176 days were not tested until nearly six months after they were removed. Since the control gave a germination of only about 50 per cent at that time, it indicates that storing in liquid air is not injurious, and it even may be beneficial in retarding normal aging of the seeds. If life is a dynamic

TABLE II  
EFFECT OF TIME OF COOLING IN LIQUID AIR ON  
GERMINATION OF SWEET CLOVER SEEDS

| TIME IMMERSSED IN LIQUID AIR | NUMBER TESTED | PERCENTAGE GERMINATION |
|------------------------------|---------------|------------------------|
| 0.....                       | 100           | 64                     |
| 2 minutes.....               | 100           | 80                     |
| 35 days.....                 | 50            | 76                     |
| 35 days.....                 | 50            | 84                     |
| 90 days.....                 | 50            | 72                     |
| 176 days.....                | 50            | 74                     |

process which obeys the laws of chemical reactions, then these reactions take place at an enormously reduced rate at this temperature. If the usual temperature coefficient of between two and three for a  $10^{\circ}$  temperature change holds over this range, a simple calculation will show that their reactions are reduced to between one-millionth and one-billionth of their velocity at normal temperatures. For seeds at this temperature, a thousand years are but a day so far as the life processes are concerned.

2. EFFECT OF REPEATED COOLING IN LIQUID AIR.—It was thought that if the rapid cooling to these low temperatures was causing any injury to the seeds, this would be increased by repeated cooling and warming. To test this, samples of Grundy County sweet clover, giving a germination of about 24 per cent for the untreated seeds, were repeatedly cooled and warmed as rapidly as possible by putting them into small ( $2 \times 0.5$  inches) test-tubes. These were immersed directly in the liquid air and then the seeds were warmed by pouring them out in a thin layer on an aluminum plate. After a

single treatment the germination was raised to 85-90 per cent; after five such treatments the germination was 92 per cent; and after twenty treatments the germination was 85 per cent (50-seed samples).

To make an even more drastic test, some alfalfa seeds in a small test-tube were covered with water and the test-tube then immersed in liquid air. After the seeds and ice had reached the liquid air temperature, the test-tube was removed and the ice allowed to melt. The seeds were then removed from the water and dried. When put on the moist filters they showed 100 per cent germination, but during germination the seed coats cracked or broke in several places, showing that they had been weakened by the treatment.

TABLE III  
EFFECT OF FREEZING IN LIQUID AIR ON CHANGE OF GERMINATION  
ON STORING

| SEEDS                      | PERCENTAGE GERMINATION |            |             |               |
|----------------------------|------------------------|------------|-------------|---------------|
|                            | Initial                | Six months | Nine months | Twelve months |
| Alfalfa, control.....      | 50                     | .....      | 72          | .....         |
| Alfalfa, treated.....      | 95                     | .....      | 92          | .....         |
| Sweet clover, control..... | 47                     | 41         | .....       | 40            |
| Sweet clover, treated..... | 70                     | 70         | .....       | 74            |

3. CHANGE OF GERMINATION ON STORING AFTER FREEZING IN LIQUID AIR.—Since many of the treatments now in use to increase germination of impermeable seeds also injure their keeping power, storage tests were run on treated and untreated seeds to see whether freezing also injured the keeping power. The tests have been run for a year without showing any great deterioration of the seeds. The results of a typical experiment with alfalfa and sweet clover seeds which had been frozen in liquid air are shown in table III.

The low germination of the sweet clover is due to the presence of many immature seeds in this particular lot. The seeds were kept in the laboratory in paper envelopes or in flasks closed with cotton so that the air could reach them, to duplicate the usual storage condition as closely as possible. The results showed that freezing in liquid air has no appreciable effect on the keeping power of the seeds.

In some germination tests, both on filter papers and in soil, it was found that the freezing not only increased the germination, but it also stimulated the growth of the seedlings. This may be due simply to the fact that water reaches the embryo more readily, but it is also possible that the liberation and the distribution of the growth enzymes are accelerated by the freezing.

## II. EFFECT OF FREEZING SEEDS IN CO<sub>2</sub> SNOW

Some of the beneficial effects of liquid air on germination of seeds can be produced by the less intense freezing obtained with solid carbon dioxide. The results of the test on dry seeds are given in table IV, where treatment I consisted in cooling with the CO<sub>2</sub> snow for 10 minutes, and then rapidly warming the seeds; while treatment

TABLE IV  
EFFECT OF FREEZING IN CO<sub>2</sub> ON GERMINATION

| SEEDS                                | PERCENTAGE GERMINATION |             |              |
|--------------------------------------|------------------------|-------------|--------------|
|                                      | Control                | Treatment I | Treatment II |
| Alfalfa, Idaho.....                  | 62                     | 86          | 87           |
| Alfalfa, Dakota.....                 | 61                     | 70          | .....        |
| Sweet clover, Grundy Co., Lot 1..... | 14                     | 22          | .....        |
| Sweet clover, Grundy Co., Lot 2..... | 53                     | 55          | 54           |
| Sweet clover, common, Lot 1.....     | 63                     | 72          | 76           |
| Sweet clover, common, Lot 2.....     | 58                     | 68          | .....        |

II consisted in cooling the seeds, and then letting them slowly warm within a Dewar flask.

It is seen that there is comparatively little change in the germination of clover seeds with this treatment, but the germination of alfalfa seeds is very distinctly improved. The seeds may be given a superficial coating of moisture before freezing, or they may be frozen repeatedly with beneficial effects on the germinability and no bad effects on the keeping power, as is shown by table V.

Freezing these seeds in liquid air raised the germination to 98 per cent. These results show that even repeated freezing in CO<sub>2</sub> snow under drastic conditions does not appreciably affect the keeping power of the seeds. Another lot of alfalfa gave an initial test of 60 and 85 per cent for the control and the treated samples respectively, and twelve months later the germination test of the same samples



was 92 and 94 per cent respectively. In this case the untreated seeds lost their impermeability on standing, but even here the treatment had no injurious effect on the seeds.

### III. FREEZING TO $-20^{\circ}$ C.

Some lots of alfalfa had their germination raised about 12 per cent by treatment, but this was only one-third of the effect produced by freezing the same seeds in solid  $\text{CO}_2$ . Clover seeds were not affected by freezing to  $-20^{\circ}$  C.

TABLE V  
EFFECT OF FREEZING ALFALFA SEEDS IN SOLID  $\text{CO}_2$  UNDER  
VARIOUS CONDITIONS

| TREATMENT                            | PERCENTAGE GERMINATION |            |            |
|--------------------------------------|------------------------|------------|------------|
|                                      | Initial                | Two months | Ten months |
| 1. Control.....                      | 53                     | 60         | 72         |
| 2. Frozen once.....                  | 86                     | 96         | 90         |
| 3. Frozen five times.....            | 98                     | 100        | .....      |
| 4. Seeds wet, then frozen once.....  | 92                     | 90         | 88         |
| 5. Seeds wet, frozen four times..... | 99                     | 92         | 96         |

### IV. SPECIFIC HEAT OF ALFALFA SEEDS

Because of the possible practical application of these results, experiments were carried out to determine the specific heat of the seeds down to  $-190^{\circ}$  C. This was done in two ways. In the first method a 500 cc. Dewar flask, half filled with fresh liquid air, was placed on a large analytical balance, and the weight of the flask and air was recorded every minute for 15 minutes. Then a weighed amount of alfalfa seeds was poured into the flask and when they had cooled off, the weight was again recorded every minute. The weight was then plotted against the time, and by extending the first and last curves to the time the seeds were added, the weight of liquid air evaporated by the seeds could be determined. The average of several experiments showed that 5 gm. of alfalfa seeds would evaporate 4.3 gm. of liquid air. Since the latent heat of vaporization of liquid air is 50 cal./gm. (9), 1 gm. of seeds give up  $4.3 \times 50 \div 5$ , or 43 cal. in cooling from  $20^{\circ}$  to  $-190^{\circ}$  C., so their average specific heat over this range is  $43 \div 210$ , or 0.21 cal./degree.

This value was checked by measuring the volume of air evapo-

rated by a known weight of seeds. A Dewar flask containing liquid air was tightly closed by a 3-holed stopper. One tube through this stopper went to a large gas burette, a second was connected by a rubber hose to a test-tube containing a weighed quantity of seeds at room temperature, and the third tube had a stopcock opening to the atmosphere. To make a determination of the specific heat, the stopcock was closed so that the air which vaporized in the Dewar flask had to go into the gas burette, and the volume of air in the burette was recorded every few minutes to get the normal rate of vaporization. After this was obtained the test-tube containing the seeds was inverted so that the seeds fell into the liquid air, and the test-tube was then returned to its original position. The transfer of the seeds thus took place without opening the system or changing its volume. The volume was again read every few minutes to get the final rate of vaporization. By extrapolating the two volume-time curves to the time at which the seeds were added to the liquid air, the volume of air evaporated by the seeds was obtained. The average of four determinations gave 700 cc. of air at room temperature and pressure evaporated by 1 gm. of seeds. Taking the weight of air under these conditions as  $1.2 \cdot 10^{-3}$  gm. per cc., 1 gm. of alfalfa seeds vaporized 0.84 gm. of liquid air, which gives the seeds a specific heat of 0.20 cal./degree. This checks the other value rather well.

In these experiments no attempt was made to utilize the cooling effect of the vapors coming from the liquid air. If one takes the molal specific heat of air between  $-190^{\circ}$  and  $20^{\circ}$  C. as 6.5 cal. per degree, then the heat absorbed when 1 gm. of air warms from  $-190^{\circ}$  to  $20^{\circ}$  C. is  $6.5 \times 210 \div 30 = 47$  cal., which is more than the heat absorbed in vaporizing 1 gm. of liquid air.

If this could be used in cooling the seeds it would reduce the amount of liquid air required by about one-half, but even under these conditions it would hardly be profitable to use liquid air to increase the germination of clover seeds commercially, since each pound of seeds would require from one-half to one pound of liquid air. However, when dealing with special experimental lots of seeds, where the ordinary procedure would be to scratch or prick each seed by hand, this method of freezing may be found very useful and convenient. Because of the high latent heat of vaporization and the

low cost of solid  $\text{CO}_2$ , it might be not only convenient but also very profitable to use this refrigerant to freeze lots of alfalfa which contain a high percentage of hard seeds.

### Discussion

The increased germination obtained after freezing clover and alfalfa seeds at very low temperatures is probably due to the formation of very tiny cracks in the impermeable membrane. This process seems to depend on at least two factors, making the membrane brittle by the cold and at the same time exerting forces tending to burst or warp it, due to the different coefficients of expansion of different parts of the seed. The extent of these forces will be determined by the size, shape, structure, and composition of the seed, the amount of water in it, and the rate, uniformity, and amount of the cooling. Some idea of the forces involved may be obtained from the fact that when castor beans or flax seeds are immersed in liquid air and then warmed rapidly and non-uniformly by placing them on a warm metal plate, they often explode with a force sufficient to rip off the outer seed coats and send the seeds flying a distance of 5-10 feet. Any treatment which magnifies these forces, such as increasing the temperature drop, either through the use of a colder refrigerant or starting with the seeds at somewhat above room temperature, tends to increase the effectiveness of the treatment. Other things, such as repeated freezing, or giving the seeds a superficial coating of moisture before freezing also helps, but their effects seem to be less than that due to cooling the dry seeds to  $-180^\circ \text{C}$ .

The fact that even repeated freezing to  $-80^\circ \text{C}$ . and rapid thawing of seeds having a superficial coating of moisture do not injure their keeping power shows that the changes produced are very minute. It seems probable that the permeability of the seed coat for oxygen is not changed much by this treatment, but it would be interesting to see whether intensive freezing destroyed the dormancy of cocklebur seeds, which is known to be due to the presence of a membrane impermeable to gases. It is possible that seeds which normally require alternating temperatures for germination might grow if kept at a constant temperature after they had been given a sufficient intense freezing. One would not expect seeds having a

dormancy due to an embryo condition to be made germinable by this treatment, and it was found that dormant belladonna and *Hyoscyamus* seeds were not made germinable by freezing in liquid air.

Since the dormancy of most alfalfa seeds can be broken by freezing around  $-80^{\circ}\text{C}$ ., and temperatures in this range can be produced very cheaply by the use of solid  $\text{CO}_2$ , liquid ammonia, etc., it may be worth while to give this treatment to all lots of alfalfa which contain many impermeable seeds.

### Summary

1. Dormancy of seeds due to an impermeable seed coat may often be broken by cooling the seeds to very low temperatures.

2. Impermeable sweet clover and alfalfa seeds may be made permeable without injuring, by freezing the air-dry seeds in liquid air ( $-190^{\circ}\text{C}$ .).

3. Sweet clover seeds were not injured by keeping them in liquid air for nearly six months (175 days); and rapidly cooling seeds down to  $-190^{\circ}\text{C}$ . twenty times, followed each time by a very rapid warming, did not injure them.

4. Cooling to  $-80^{\circ}\text{C}$ . caused most of the impermeable alfalfa seeds to become permeable, but this treatment had very little effect on impermeable sweet clover seeds.

5. Freezing air-dry seeds to very low temperatures had no appreciable effect on their keeping power.

6. The average specific heat of air-dry alfalfa seeds between  $20^{\circ}$  and  $-190^{\circ}\text{C}$ . is about 0.21 cal. per degree.

7. The process of destroying the impermeability of alfalfa seeds by intense freezing appears to be a practical one for large-scale operations.

The writer wishes to express his appreciation to Professor F. DANIELS for the constant inspiration he has furnished throughout the work, and to the members of the botany and agronomy departments for the help they have given.

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## LITERATURE CITED

1. BROWN, H. T., and ESCOMBE, F., Effect of liquid air temperature on the germination of seeds. *Proc. Roy. Soc.* 62:160. 1897-8.
2. CROCKER, WM., The mechanics of dormancy in seeds. *Amer. Jour. Bot.* 3:99-120. 1916.
3. DAVIES, P. A., High pressures and seed germination. *Amer. Jour. Bot.* 15:149-155. 1928.
4. GRABER, L. F., The effect of scarifying on the keeping power of alfalfa seed. *Jour. Amer. Soc. Agron.* 14:298-302. 1922.
5. HARRINGTON, H. I., The agricultural value of impermeable seeds. *Jour. Agric. Res.* 6:761-796. 1916.
6. LUTE, A. M., Impermeable seeds of alfalfa. *Bull. no. 326. Colo. Exp. Sta.* pp. 34. 1927.
7. MIDGLEY, A. R., Effect of alternate freezing and thawing on the impermeability of alfalfa and dodder seeds. *Jour. Amer. Agron. Soc.* 18: (12): 1087-1098. 1926.
8. SCHMIDT, D., Work on the hard seed problem. *Seed World* 19: (1):9. 1926.
9. SMITHSONIAN PHYSICAL TABLES. 7th ed. 231. 1920.
10. STAKER, E. V., The effect of dry heat on alfalfa seed and its adulterants. *Jour. Amer. Soc. Agron.* 17:32-40. 1925.
11. STEINBAUER, G. P., Differences in resistance to low temperatures shown by clover varieties. *Plant Physiol.* 1:281-286. 1926.
12. STEWART, GEO., The effect of scarification and dry heat on alfalfa. *Jour. Amer. Soc. Agron.* 18:743-760. 1926.

# SPERMATOGENESIS IN COLEOCHAETE SCUTATA

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(WITH PLATES V, VI)

## Introduction

This paper is a report of the continuation of the work outlined in a former paper (5). PRINGSHEIM (4), OLTMANNS (2), and JOST (1) have studied the spermatogenesis of one or more species of *Coleochaete*. As early as 1860, PRINGSHEIM described the spermatogenesis of *C. scutata*, so far as the process could be observed in whole mounts. So far as I have ascertained, no sections of the thalli bearing antheridia have ever been studied. The purpose of the present paper is to report the results of a study of such sections, together with a few observations made upon surface views.

## Material and methods

The major part of the material for this work was collected at Miller, Indiana. The collections were made at one- and two-week intervals from June 18 until October 30, 1927. Some information, however, was obtained from material collected at weekly intervals from a lagoon in Washington Park, Chicago, beginning July 6 and continuing until November 30, 1926, after which monthly collections were made until April, 1927. The best developed thalli were found on *Typha* leaf sheaths of the previous year's growth. The material collected in Washington Park was on *Sagittaria*, *Typha*, *Nymphaea*, and *Castalia*; none was found on sticks, stones, or other aquatic plants than those mentioned. In collecting about Madison, Wisconsin, however, *C. scutata* has been found also on the leaf sheaths of *Sparganium* and on the leaves of *Potamogeton*.

The material differed in no way from that described in the previous paper (5), except that a few sections were stained with Flemming's triple stain. The greater part of the material was stained in Haidenhain's iron-alum haematoxylin. Sections were cut 3-7  $\mu$  in thickness.

### Spermatogenesis

TIME OF FORMATION.—When the first collection of material was made, June 18, 1927, a number of thalli showed that the first division of a vegetative cell in preparation for the formation of antheridia was complete. At this time female plants were found in which the zygotes were completely covered with dark brown cells. These zygotes were almost if not quite mature; hence antherozoids must have been produced much earlier than the collection date. Eggs in some of the thalli had disintegrated, probably because of lack of fertilization. In all the material collected at Miller, the number of abortive eggs was high in comparison with the number of mature zygotes. Very few thalli produced zygotes, and those having them had very few.

In the material collected in Washington Park, antheridia and oogonia did not begin to appear until about the middle of September. Since the two places are only a few miles apart, it seems surprising that antheridia and oogonia should be produced in one location so much earlier than the other.

The thalli producing antheridia on June 18, 1927, were all found on *Typha* leaf sheaths of the previous year's growth. As soon as the ice melts in the spring, zoospores begin to form in the thalli that have over-wintered. This time of formation of zoospores comes long before the new vegetation begins to appear. *Typha* is more resistant to the forces bringing about its decay than is *Sagittaria*, hence the old *Typha* stems wrapped in their leaf sheaths are rather well preserved at this time. Zoospores come to rest upon these leaf sheaths, and thalli begin to develop. This early development gives them the start over those that are later formed on the fresh living plants. Since these early thalli reach maturity before the others, it is natural for them to begin to form antheridia and oogonia before those developing later.

According to WEST and FRITSCH (6), *Coleochaete* produces antheridia and oogonia in England from May to June. OLTMANNS reports sexual reproduction for the "Titisee" region from September to October. He says (3) that the time of sexual reproduction depends upon the locality, implying that differences in temperature are the effective factors. In the region about Chicago, however, no such

range of temperature could account for the two months' difference in time in the production of antheridia. Miller is only thirty miles from Chicago; hence there could be no such difference of temperature as one finds between England and Germany.

Antherozoids ready to be shed, as well as cell walls from which they had escaped, were found in the material collected June 27, 1927. The number of antheridia increased until the middle of September, then gradually declined until the latter part of October. A few were found as late as October 30 (1927).

No swimming antherozoids were observed; hence the time of their escape was not determined. The time of day at which the division by which antheridia are formed takes place was likewise not determined. Material was killed at intervals from 7:00 A.M. to 6:00 P.M. (fig. 1), however, and only prophases were found. From this fact one would infer that the division took place between 6:00 P.M. and 7:00 A.M.

PLACE OF FORMATION.—Although JOST reports that antheridia and oogonia were found on the same plant of *C. scutata*, such a condition has not been found on material used in the present investigation. Hundreds of disks were examined; but all, if bearing sexual organs at all, were found to be either antheridial or oogonial. So far as is known, all other species of *Coleochaete* produce both kinds of sex organs on the same plant, and it would not be surprising to find an occasional disk of this species doing the same.

The first antheridia formed are usually produced about halfway between the center and the circumference of the disk. According to PRINGSHEIM, a cell or a small group of cells begins division to form antheridia: first those adjacent to the dividing cells on a circular band whose center is the center of the disk divide, next those adjacent, and so on round the band. This continues until a band, more or less complete, extends around the disk. He reports the formation of other bands in the same thallus, all of which are formed in somewhat the same manner. My observations confirm his, save that instead of one group of dividing cells there may be several; and the band seems to be more interrupted than he indicates. Often sectors from one to four cells in width separate the bands into many groups. Scattered all through the groups there are cells that remain un-



changed. Often these are the cells that have borne hairs, but sometimes they are not. The band of antheridia seems to be wider and more irregular than PRINGSHEIM's description would indicate.

After the first band of antheridia has been formed, other bands arise. These may be either peripherally or centrally located with regard to the first band. A disk has been observed which showed a cell next within a peripheral one dividing to form antheridia (fig. 1 *1d*). Fig. 16 shows an antherozoid just ready to begin its escape. Only one cell separates the antheridium from the nearest peripheral cell. No peripheral cells have been found forming antheridia, and it is here that one finds a wide departure from the condition in other species of the genus, all of which form antheridia from terminal cells. In another thallus, a cell between the first band of antheridia formed and the peripheral cells had formed an antheridium before any other cell outside the first zone had gone through even the first division (fig. 4*a*). More often, however, an entire zone will have passed through the first division to form antheridia before the second division in the process of antheridial formation begins.

FIRST DIVISION.—The increase in both number and size of the cells of a disk is limited to the peripheral cells, as was noted by PRINGSHEIM, and as has been confirmed by other workers, including the writer (5). Once a cell ceases to be peripheral it never divides again, save for one of two reasons, so far as is known; for at just what time and for what reasons the thallus becomes two cells thick has not been determined by any of those who have studied the different species of this genus. Any cell in the interior of the disk may divide as the result of an injury to the adjacent cells, as previously reported (5); it may also undergo division in preparation for the formation of antheridia. This will hereafter be called the first division. The first wall formed in the latter case may be either radial or tangential. If either condition predominates it would seem to be the radial, although no attempt has been made to determine this point. A given sector may show only radial or only tangential divisions, although more often the two are indiscriminately mixed (figs. 6, 12). Fig. 2 *1d* shows a radial division which forms two rectangles from the outer surface wall; while fig. 3 shows the first wall dividing the outer wall of the cell into what in surface view appears as two nearly

equal triangles, reminding one of the divisions of the androgone mother cells in various *Marchantiales*. This first wall may (as it usually is) be formed at right angles to the plane of the outer wall of the dividing cell, or it may form an angle with the outer wall (figs. 7, 8). The angle thus formed may be so acute as to leave doubt as to whether the cell thus formed is an antheridium, or a cell resulting from the first division of a vegetative cell to form the two antheridia mother cells (fig. 7*d*). The two cells formed by the first division may thus be very unequal or they may be closely similar in shape and size (figs. 7, 9). If the thallus is two cells thick, the outer cell only is divided, the inner one, next the substratum, taking no part in antheridial formation (fig. 8). No case in which both had been divided has been observed.

SECOND DIVISION.—This division, which hereafter will be referred to as the second, which PRINGSHEIM thought to be the final one in antheridia formation, divides each of the daughter cells formed by the first division into a small antheridia and a large cell. The two second divisions may occur simultaneously, as shown in the lower cells of figs. 1 and 2, or they may occur at different times, as shown in fig. 4. Unlike the wall formed after the first division, the new partition wall always makes an acute angle with the surface plane of the two cells resulting from the so-called first division, as is shown by fig. 5. Although the angle is always acute its size varies, and the resulting antheridium also varies in size; this is why one cannot be sure whether the small cell formed by the division of the right hand cell in fig. 7 is an antheridium or one of the two daughter cells formed by the first division of the ordinary vegetative cell. The first antheridium of the pair formed by division of one of the two daughter cells is always larger than the second one formed from the same daughter cell. Fig. 5 shows that more than half of the upper portion of the cell is used.

THIRD DIVISION.—Soon after the second division, a third one occurs, which will hereafter be referred to as the third division. The large sister cells of the first antheridia formed from each of the two daughter cells of the first division divide to form another antheridium and a large sister cell. This third division follows so quickly after the second as to make it almost impossible to find one antheridium

cut from the two daughter cells of the first division without the second one. The third divisions may occur at the same time in the two daughter cells of the first division, or they may occur at different times, as in the case of the second division. This third division is followed by the formation of a wall that forms an acute angle with the plane of the outer surface of the cell undergoing division, as is the case after the second division. The opening of the angle, however, is in the opposite direction to that of the angle made by the wall that is formed after the second division with the plane of the outer wall of the original cell.

The second antheridium is slightly smaller than the first, as just noted; otherwise they are similar. An antheridium at the time of formation contains a very large nucleus, a small chloroplast, and more or less dense cytoplasm. The protoplast of each antheridium becomes a single antherozoid.

When the four antheridia formed thus from one cell are viewed from the surface, as PRINGSHEIM saw them, they give the effect of one cell divided to form four (fig. 2); but a section of the disk at right angles to the surface tells an entirely different story. This view even in section is not always the same, because it depends upon whether the section is cut parallel or at right angles to the first wall laid down. If the former, a large five-sided vegetative cell with two small antheridia which have been formed by division from one of the daughter cells of the first division is shown (fig. 6). If the section is at right angles to the first wall, there are two long narrow cells with one antheridium cut from the upper end of each (figs. 8, 13). Here the antheridial wall is usually at right angles to the lateral walls of the long narrow vegetative cells (fig. 13), but the antheridial wall may form any angle with the lateral walls (figs. 8, 9). Sometimes whole sections will have the antheridial walls at right angles to the lateral walls of the vegetative cells below; at other times they may all form acute angles with these walls; while still more frequently those forming right angles and those forming acute angles will be indiscriminately mixed (figs. 6, 12, 18).

VARIATIONS.—Each of the two daughter cells formed by the first division by a second and third division normally produces two antheridial cells. Sometimes more than two cells may be formed, how-

ever; that is, a fourth division of the sister cell to the second antheridium may result in a small cell similar in shape, size, and content to an antheridium (figs. 14, 15). Other similar divisions may occur, resulting in as many as five cells that resemble antheridia in every way. Whether all these antheridia-like cells function as antheridia has not been definitely determined, because, although the outer walls were ruptured and the contents gone, this condition might have been due to cutting. The size of the cells, however, their contents, and their position would lead to the conclusion that these small cells are all antheridia producing antherozoids as in the case of the first two usually cut off. None but cells producing antheridia are ever found to have formed these small antheridia-like cells. Although not abundant, they are by no means infrequently found. Certainly this species is quite variable in all other respects, therefore it may well vary in this, forming several antheridia from each daughter cell of the first division.

**SISTER CELL.**—At the third division the sister cell of the second antheridium usually does not undergo further cell division, although in a few cases division must have occurred either after the formation of the first antheridium or at least at some time after the first division. At any rate, where one cell is usually, occasionally two cells are found. This sister cell of the last antheridium formed is a seven-sided vegetative cell. The outer wall, the one opposite the substratum, is made up of two walls, of which a section shows a cell with five walls (figs. 6, 7). In case the thallus is two cells thick, two vegetative cells occupy the space usually occupied by the one sister cell of the last antheridium formed. These cells have the usual contents; each possesses a nucleus, cytoplasm, and a chloroplast (figs. 6-9).

**CHLOROPLAST.**—All writers on this species agree that each antherozoid (only one being formed in each antheridium) is green in color, while those of the other species of this genus are colorless. Never having seen the swimming antherozoid, it is impossible for me to confirm this statement. The evidence from surface views and from sections is conflicting. From the surface view (fig. 2an) no chloroplast could be seen. Sections through antheridia soon after their formation showed a definite but small plastid in each. Other an-

theridia showed plastids that seemed to be disintegrating. These were smaller, showed no pyrenoid, were very vacuolate, and took the stain very lightly. Others showed no sign of a chloroplast. This might have been a result of cutting, but the very large nucleus was clearly visible in each case, and all zoospores showed the chloroplast closely associated with the nucleus. Numerous antherozoids which were escaping from the thallus were examined and not one showed a chloroplast (figs. 16, 17). Zoospores at this stage never fail to show chloroplasts. If the antherozoid has a chloroplast, what becomes of it? Zygotes whose chloroplasts have just begun to divide showed no sign of the presence of a chloroplast from the antherozoid.

**ANTHEROZOID ESCAPE.**—The antherozoid escapes through a pore that, from all indications, is formed in the same way as that for the escape of the zoospore. An opening is formed for the escape of each zoospore, but the two antherozoids formed in the antheridium that has been formed by the division of one of the daughter cells of the first division and in the antheridium that has been formed by the division of this sister cell of the first antheridium may escape through the same pore (figs. 7, 10). Sometimes, however, the two antherozoids formed in the manner just described may escape through different pores (fig. 12). The manner of escape corresponds exactly to that of the zoospores (figs. 16, 17) as previously described (5). That the male gametes escape in great numbers is shown by figs. 18 and 20. In section the fragments of the antheridial walls can be clearly seen but in surface view the thallus has a decidedly ragged appearance due to the presence of fragments of the old antheridial walls.

**SISTER CELL CHANGES.**—At first the outer walls of the sister cells form an acute angle (figs. 7, 12). Soon after escape of the antherozoids, the acute angle is retained and the walls are thin and delicate. Later these outer walls become gradually thicker, until they are approximately as thick as the usual surface wall of a vegetative cell. Soon the pressure of the contents eliminates the sharp angle of these two outer walls, and the two become rounded and thereafter have the appearance of one wall rather than two. These sister cells of the antheridia mother cells may form hairs like any other young cell. Sometimes these hairs are found projecting through the pores

through which the antherozoids have escaped (figs. 19, 20). In a thallus two cells in thickness, the inner cell may likewise form a hair after the escape of the zoospore from the outer cell (5).

### Discussion

Although the manner of formation of antherozoids differs widely in the various members of the Chlorophyceae, none described for other genera in any way approximates the method found in *Coleochaete scutata*. The Chlorophyceae range from the Conjugatae in which the apparently unchanged content of an ordinary vegetative cell acts as a male gamete, to the Charales which have the most complex antheridia found in any plant. Between these two extremes appear several gradations in antheridium formation. In *Ulothrix*, for example, the content of a vegetative cell divides to form a number of gametes which may or may not be differentiated into male and female. In *Vaucheria* a special cell, an antheridium, is developed for the production of antherozoids, and produces many. *Oedogonium* may have its antheridia on a male plant exactly like the female plant, on a small, few-celled plant epiphytic on the female, or on the same plant with the oogonia. In every case the antheridium is a cell exactly like the ordinary vegetative one save that it is much shorter. It is produced in exactly the same way as is the vegetative cell. In each antheridium two antherozoids are produced, or sometimes only one.

In all species of *Coleochaete*, so far as known, the antherozoids are produced in special cells. In *C. pulvinata*, *C. irregularis*, and *C. nitellarum* the antheridia develop from the terminal growing cell. These cells, the antheridia, are produced just like any vegetative cell except that they have no chloroplast, are smaller, and more pointed at the outer end. The antheridia occur in groups. One colorless antherozoid is produced from each antheridium. This method is best illustrated in *C. pulvinata* (2).

Unlike these, *C. scutata* produces its antheridia by division of an interior cell of the thallus which ordinarily would not divide again. There is no increase in size, no protusion of the cell wall, nor any other noticeable change unless the protoplasm becomes slightly more

dense. The nucleus of each daughter cell of the first division divides following the unequal division of the chloroplast; and a second cell division forms another antheridium. In each, one antherozoid is formed. The two antherozoids usually escape through a common pore, which is apparently formed by enzymatic action. This method of antheridium production seems to be unique, and would seem to place *C. scutata* next to the Charales in the complexity of the development of the antheridium.

### Summary

1. Antheridia are produced from the middle of June until the middle of October in the region in and about Chicago.
2. The cells from which bands of antheridia are formed may begin by the division of a single cell or by division of groups of cells some distance apart. The first group arises about halfway between the center and the circumference of the disk. Outer and inner bands appear later, sometimes arising almost simultaneously.
3. The first division of a vegetative cell produces two daughter cells, antheridia mother cells, by whose division antheridia are formed.
4. The second division produces an antheridium from each daughter cell formed at the first division.
5. The sister cell of the second antheridium is a large seven-sided vegetative cell, which remains after the escape of the two antherozoids from the antheridia.
6. More than four antheridia may be developed from one vegetative cell.
7. The outer wall of the sister cell of the last antheridium formed becomes rounded and thickened, until it takes on more or less the appearance of the outer wall of the ordinary vegetative cell.
8. Hairs are sometimes produced by these sister cells of the last antheridium formed.
9. Some antheridia contain normal chloroplasts, some contain chloroplasts that appear to be disintegrating, while still others show no chloroplasts at all.
10. The antherozoids formed from each of the daughter cells of the first division usually escape through a common pore.

This work was made possible by a research fellowship given by the University of Wisconsin, where the work was done under the supervision of Professor C. E. ALLEN, to whom I am greatly indebted for many valuable suggestions and criticisms.

AMARILLO  
TEXAS

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#### LITERATURE CITED

1. JOST, L., Beiträge zur Kenntnis der *Coleochaete*. Ber. Deutsch. Bot. Ges. 1895.
2. OLTMANN, FR., Die Entwicklung der Sexualorgane bis *Coleochaete pulvinata*. Flora 85. 1898.
3. ———, Morphologie und Biologie der Algen. 1:317-322. 1922.
4. PRINGSHEIM, N., Beiträge zur Morphologie und Systematik der Algen. III. Die *Coleochaeten*. Jahrb. Wiss. Bot. 2:1-37. 1860.
5. WESLEY, OPHELIA C., Asexual reproduction in *Coleochaete*. BOT. GAZ. 76: 1-31. 1928.
6. WEST, G. S., and FRITSCH, F. E., A treatise on the British freshwater algae. Cambridge Univ. Press. 1927.

#### EXPLANATION OF PLATES V, VI

All figures were drawn with the aid of the camera lucida on a level with the base of the microscope. Spencer apochromatic objective 1.5 mm. N.A. 1.30 was used. Compensating ocular 15X was used for figs. 1-4, 16, and 17, giving a magnification of 1370X; a Zeiss 4X compensating ocular was used for all other figures, giving a magnification of 1020X. All figures are reduced one-half.

The small letters on all plates have the following meanings: *a*, antheridium; *an*, antherozoid; *c*, chloroplast; *cy*, cytoplasm; *1d*, first division; *2d*, second division; *3d*, third division; *g*, granules; *n*, nucleus; *p*, peripheral cell; *po*, pore; *vc*, vegetative cell.

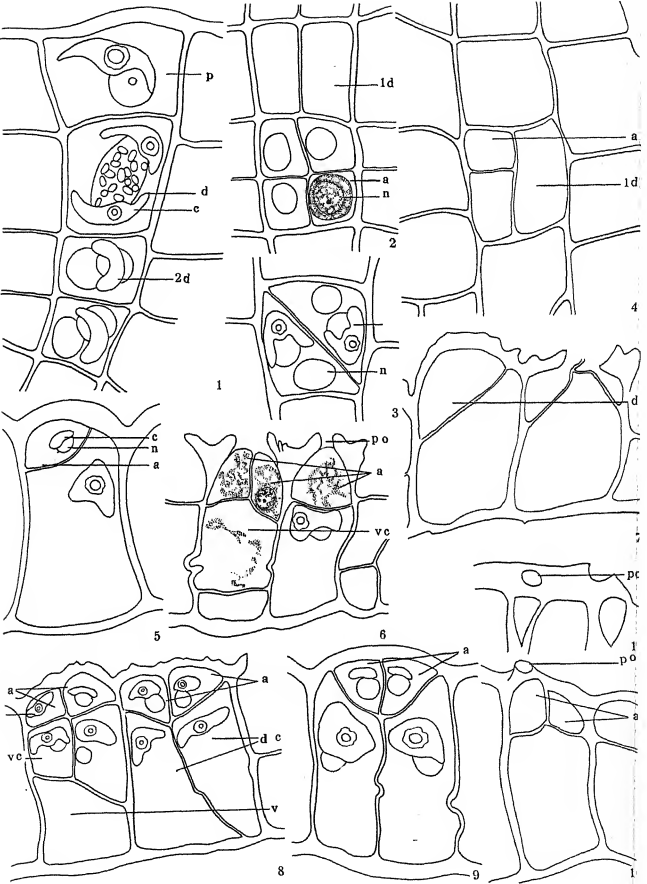
#### PLATE V

FIG. 1.—Surface view of peripheral cell, next cell in late prophase for first division to form antheridium, and third cell in from periphery showing nucleus divided but wall not yet formed.

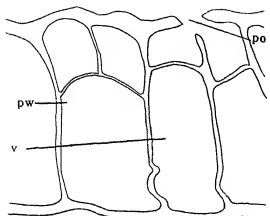
FIG. 2.—Surface view of two cells, upper showing first division, lower showing completed antheridia.

FIG. 3.—Surface view of cell showing wall formed at first division passing diagonally across cell.

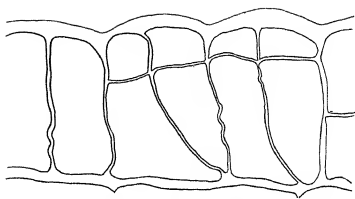




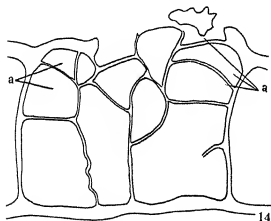




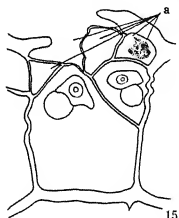
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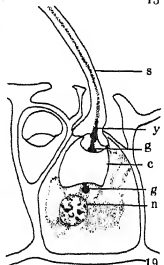
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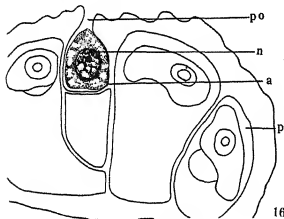
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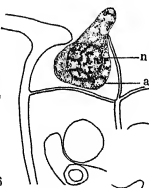
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19



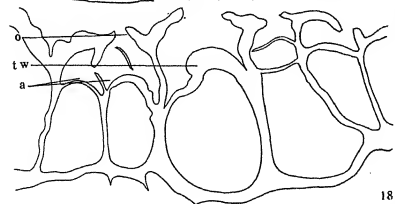
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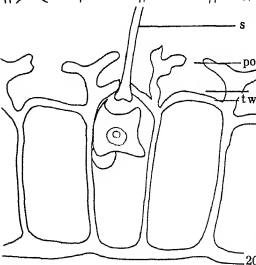
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18



20



21



FIG. 4.—Surface view of cell near outer edge of disk, showing at least one antheridium while no other cell outside the first band formed has even undergone first division.

FIG. 5.—Section through thallus at right angle to surface and parallel to first wall, showing first antheridium formed.

FIG. 6.—Section similar to that shown in fig. 5, showing second antheridium formed by division of sister cell of first antheridium.

FIG. 7.—Section showing cell at left which cannot be definitely placed as either one of the two daughter cells or as first antheridium; cell at right shows two empty antheridia with common pore and remaining vegetative cell.

FIG. 8.—Section showing in cell to left that only upper of the two cells is involved in formation of antheridia (section cut parallel to first wall formed); in this and cell to right may be seen variations in angles made by antheridial walls with lateral walls of adjacent vegetative cells; cell to right shows inequality of two cells formed by first division.

FIG. 9.—Wider variation of walls separating antheridia and vegetative cells.

FIG. 10.—Section through thallus cut just to side of pore.

FIG. 11.—Section through thallus cut at angle to surface giving small portion of surface in which pore was located; just below is empty antheridium.

#### PLATE VI

FIG. 12.—Section through thallus showing cell at left cut parallel to first wall, while one at right is cut at right angles to first wall; one at right shows common pore for two antheridia which have had their origin in different daughter cells.

FIG. 13.—Section showing number of cells cut at right angles to first wall; some show antheridia formed from them while others do not.

FIGS. 14, 15.—Sections of cells showing more than two antheridial-like cells formed from one daughter cell and its descendants.

FIG. 16.—Section of thallus showing antherozoid ready to escape before third division has taken place; only one cell between it and peripheral cell.

FIG. 17.—Section showing two antherozoids passing out of pores.

FIGS. 18, 20.—Sections through thalli after escape of antherozoids, showing remains of old antheridial walls and thickened outer wall of remaining vegetative cells.

FIGS. 19, 20.—Sections showing hairs formed by vegetative cells left after escape of antherozoids; fig. 19 shows section through hair while fig. 20 shows section cut just to side of hair.

## FLOWER PRODUCTION BY ORCHID GROWN NON-SYMBIOTICALLY

LEWIS KNUDSON

(WITH THREE FIGURES)

### Introduction

In a number of papers previously published (6-9), issue has been taken with the view advanced by BERNARD (1), BURGEFF (3), and others that the orchid fungus is necessary for germination. In these various papers evidence was presented demonstrating that the germination of orchid seeds is dependent on an outside source of organic food. If a utilizable sugar is provided germination proceeds readily; if starch is provided there is no germination unless some means is provided for converting the starch into sugar. This may be accomplished by supplying the orchid fungus, or by the use of other fungi which will digest the starch and provide an appropriate hydrogen-ion concentration without at the same time growing so luxuriantly as to prevent growth of the orchid embryos. In the earlier papers the view was emphasized that the action of the orchid fungus is purely external, and therefore of no particular significance to the orchid embryo unless starch is available or other digestible food is supplied.

It is not necessary here to summarize the evidence for the non-symbiotic view. A recent paper (9) discussed the evidence for and against obligative symbiosis. The present paper is concerned primarily with the view held by some that obligative symbiosis is a requisite for flowering of the orchid plant.

In a review of my first paper, COSTANTIN and MAGROU (5) intimated that seedlings produced by the non-symbiotic method would not flower. COSTANTIN (4) referred to this in a second paper, but later BULTEL (2) showed that plants produced from seedlings germinated asymbiotically would flower; and my results (9) were confirmatory. In the development of the orchid plants to the flowering stage, infection of the roots occurred both with BULTEL's plants as

well as mine. Referring to BULTEL's work, COSTANTIN (4) intimates again that the flowering of these plants was due to infection of the roots by the orchid fungus, and states that the fungus probably contributes a vitamine which is necessary for reproduction. In a review of my second paper COSTANTIN (4) invites me to produce flowers on plants raised asymbiotically, with the intimation that this is not possible.

It was hoped that seedlings transplanted and grown in osmunda fiber would develop without infection, since the plants were maintained in a greenhouse not previously used for orchids. MERCIER'S (10) work showing the wide distribution of the orchid fungi showed clearly the improbability of maintaining orchid plants free of the fungus when the plants are grown in the open. Fortunately another experiment had been devised to test the theory proposed by COSTANTIN that an orchid plant would not flower without fungus infection. In this particular experiment an attempt was made to develop an orchid plant to the flowering stage under pure culture conditions.

#### Experimental method

Seeds of a *Laelia-Cattleya* hybrid were sown in a tube culture, using the methods previously described (6). The culture solution used was Pfeffer's, the phosphate supplied being monobasic potassium phosphate. To this solution was added 2 per cent glucose. The agar content was 1.5 per cent and the reaction of the sterilized culture medium was pH 6. The initial sowing was made on October 20, 1920. Owing to the high pH value growth was relatively slow, and the seedlings were left in the tube until August 4, 1921. By this time the culture medium was reduced to about one-half of the original volume, and the seedlings had developed two small leaves with the first root just beginning to appear. On August 4, 1921, a few seedlings were transferred to a 250 cc. Erlenmeyer flask containing solution B with 1.5 per cent agar but without any sugar. The pH of this solution was 5.8. Growth on this medium was again relatively slow, and the leaves were yellowish green, due to the low availability of iron. On December 22, 1923, a single plant with several leaves 2 inches in height was transferred carefully to a 12-liter flask containing 4 liters of solution B of one-half concentration, and

adjusted by the addition of N/10 hydrochloric acid to a pH value of 4.9. The agar content was 1.5 per cent. Previous experiments had revealed this as the most favorable value with solution B. It may be mentioned that this pH value permits the availability of iron by maintaining it in solution. It should be added that sugar was not added to the culture medium.

The 12-liter flask with the culture medium was sterilized by autoclaving, and in transplanting the seedling every precaution was taken to insure sterile conditions. After transplanting, the flask was taken to the greenhouse where it was placed in a shaded chamber to prevent any burning of the inclosed plant by the sun.

### Results

New growth quickly appeared, and after 8 months in the large flask the plant had the appearance shown in fig. 1. From December, 1923 to June, 1927 the culture remained free of any organisms; thereafter for some reason not apparent certain contaminations appeared. The first apparent contamination was an alga, a species of *Chlorella*, and later appeared a small colony of a moss and a contamination resembling *Penicillium*. The orchid plant continued to thrive until late in 1928, developing well (fig. 2), with a very abundant root system. The roots for the most part penetrated the agar medium and some extended to the bottom of the flask. During the entire period (December, 1923 to November, 1928) no additions were made to the culture medium, and not more than 2 liters of water had evaporated at the end of this time.

Concurrently with the transfer of a plant from the Erlenmeyer flask to the large one, about twenty-five seedlings were transplanted to pots containing osmunda fiber, as is usual in practical procedure. These plants developed in a manner comparable with the one in the flask. Of the initial seedlings transplanted in pots twenty survived. Of these, five flowered from October to December, 1927; the remaining ones flowered in October, November, and December, 1928. The roots of five of these plants were examined in 1927 and 1928 and the fungus was found to be present.

The single plant in the large flask finally flowered in November, 1928 (figs. 2, 3). The flower stalk produced two flowers which were



normal in every respect. The flower stalk, however, was less rigid than is usual for plants grown in the open. This no doubt was due partly to the high humidity prevailing in the flask and partly to the shaded condition under which the plant developed. The flower not

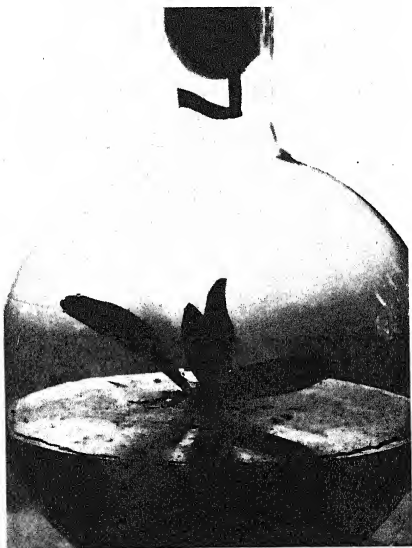


FIG. 1.—Plant  $3\frac{1}{2}$  years old; reduced to one-third

only structurally but otherwise was similar to the flowers produced in the open on plants with infected roots. Unfortunately, before photographing the plant the flask was left for three days in the laboratory, and either because of fumes or the dry atmosphere, the petals began to droop just before the photograph was made.

In regard to other features, perhaps the outstanding one was the

very extensive development of the root system (fig. 3). Of a total of twenty-seven main roots produced only two had died. The others as well as the lateral roots were in a healthy condition. As would be expected, the roots were all slightly green or greenish in color, and microscopic observation revealed the presence of chloroplasts in



FIG. 2.—Plant flowering in November, 1928; reduced to one-fourth

every root. The viability of these roots is in striking contrast to that of roots growing in osmunda fiber in pots.

At the conclusion of the experiment the flask was broken so that the culture medium could be examined and the plant removed for further observation. Immediately ten small samples of the agar medium were removed from different areas at the surface of the agar and platings made. Similarly eight samples of agar were re-

moved from the agar mass at a depth of about 2 cm. from the surface. A potato agar medium was used as the culture medium for plating. The petri dishes were incubated at 26° C., and there developed in the



FIG. 3.—Details of plant removed from flask

lot two forms of *Penicillium*, one *Fusarium*, and two forms of yeast with several bacterial colonies apparent. There was no evidence of the presence of the orchid fungus.

Microscopic examination of the culture medium was made immediately following the removal of the plants. The apparent contaminations were a species of *Chlorella* which practically covered the surface. There was in addition a colony of moss. There was noted also some fungus growth but none of the hyphae observed had the characteristic appearance of the orchid fungus.

To establish the absence or presence of the fungus organism in the roots, freehand sections were made of every main and secondary root of the orchid plant. For the main roots, sections were made about 0.5 cm. behind the tip and at regular intervals up to the base. Three or four regions were examined from every main root, and in like manner three regions were examined in every secondary root. A total of sixty-five roots was examined. Four or five sections were made in each case and every one carefully examined. In no case was there any evidence of infection. As stated previously, all of the roots were green or slightly green in color, and the cortical cells of all roots had chloroplasts. This is of some significance as it has been the general observation that roots containing chloroplasts are not invaded by the orchid fungus.

### Conclusion

A consideration of the evidence here presented must lead to the conclusion that the symbiotic condition is not obligative either for germination or for growth and flower production. The contention may be made that the plant was grown under purely artificial conditions with a relatively high concentration of nutrients, and that organic matter may have been supplied because of the contaminations. It is true of course that the seedling was grown for 10 months in the presence of sugar, and COSTANTIN has several times stated that the high concentration of sugar in its effect is similar to those chemical substances effective in artificial fertilization. This argument has been adequately answered in a previous paper (9), however, and needs no further consideration. The contention may be made also that organic matter may have been available to the orchid plant as a result of the contamination that occurred during the last 18 months of growth. The hydrolysis of some of the agar would yield galactose, but unpublished data show that this sugar is not utilizable by the orchid plants. Some sugar may have resulted from

the decomposition of *Chlorella*, but the amount would have been very slight or the growth of *Penicillium* and *Fusarium* would have been markedly increased and not just visible, as was the case. It may be concluded that no more organic matter was available for the orchid grown in the flask than is available for epiphytic orchids growing under natural conditions, where decay of moss, lichens, and bark is constantly in progress. In conclusion it may again be stated that obligative symbiosis is not requisite either for germination or for flower production.

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#### LITERATURE CITED

1. BERNARD, NOËL, Evolution dans la symbiose. Ann. Sci. Nat. 9:1-296. 1909.
2. BULTEL, Les Orchidées germées sans Champignons ont des plantes normales. Rev. Hort. p. 125. 1926.
3. BURGEFF, HANS, Die Wurzelpilze der Orchideen, ihre Kultur und ihr Leben in der Pflanze. Jena. 1909.
4. COSTANTIN, J., La vie asymbiotique des orchidées. Ann. Sci. Nat. Bot. Series 10. 8: i-xiv. 1926.
5. COSTANTIN, J., and MAGROU, J., Applications industrielles d'une grande découverte Française. Ann. Sci. Nat. Bot. Serie 10. 4: i-xxxiv. 1922.
6. KNUDSON, LEWIS, Non-symbiotic germination of orchid seeds. BOT. GAZ. 73:1-25. 1922.
7. ———, Further observations on non-symbiotic germination of orchid seeds. BOT. GAZ. 77:212-219. 1924.
8. ———, Physiological study of the symbiotic germination of orchid seeds. BOT. GAZ. 79:345-379. 1925.
9. ———, Symbiosis and asymbiosis relative to orchids. New Phytol. 26: 328-336. 1927.
10. MERCIER, A., Les Orchidées du Brésil, La Forêt. Rev. Sci. 64: p. 244. 1926.

## OXIDATION-REDUCTION INDICATORS AS A MEANS OF DETERMINING OVERHEATING IN WALNUTS DURING DEHYDRATION<sup>1</sup>

A. R. C. HAAS

Among the chief advantages of dehydrating walnuts by artificial heat over sun-drying are the improvement of quality and the early marketing because of rapid curing. As found by BATCHELOR and others (1), the highest temperature at which walnuts can be dehydrated with safety is 110° F., although with very moist nuts 105° F. was found to be safer. To reduce the time and cost of dehydration, the nuts are dried at a temperature as close as possible to the so-called "critical" temperature beyond which they cannot be heated without danger of injury. Since dehydrators are sometimes operated by growers who do not realize the consequences of overheating, the product in some cases becomes rancid by the time it reaches the consumer. This involves not only a financial loss, of course, but also a loss of reputation to the marketing association. If a method could be found by which overheated nuts might be detected before they are mixed with other nuts, the annoyance and financial loss due to overheating would be reduced to a minimum.

In this paper a method is described which the writer believes is reliable and practical. Preliminary tests gave indications that some method based on oxidation and reduction would be a most promising one. While these studies were under way an article by HIBBARD and MILLER (3) appeared in which it was pointed out that seeds of varying degrees of viability reduce very dilute solutions of potassium permanganate. The indicator, however, proved to be too rapidly reduced by walnut material to be of use in the present investigation, but tests with other oxidation-reduction indicators confirmed the claim of HIBBARD and MILLER that the underlying principle involved is oxidation-reduction.

In the tests to be described, the oxidation-reduction indicators developed by CLARK and others (2) furnish the basis for the method.

<sup>1</sup> Paper no. 141, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

The principles involved are discussed by TAYLOR (6) and NEEDHAM and NEEDHAM (5).

The method briefly is as follows. Walnut kernels from two sources (control nuts used as a standard, and the nuts suspected of being overheated) were finely mashed and mixed in mortars. Samples of equal weight were weighed and placed on small pieces of paper. The samples in each case were added to test-tubes containing a known amount of buffer solution (about pH7) containing the oxidation-reduction indicator. After an arbitrarily chosen period the tubes were shaken and the contents filtered. The colors of the solutions in corresponding tubes were compared and the sample showing the greater degree of reduction considered overheated with respect to the sample chosen as standard.

In the method used by the writer six walnuts were selected from a lot known to have been dehydrated at 110° F. for 24 hours. This standard was chosen arbitrarily as no studies were undertaken to establish a fixed standard applicable to practice. Six walnuts were also selected from the lot of nuts supposedly overheated. Only those nuts were selected which were well sealed, the kernel free from shrivel, mold, etc., and as light in color as the lot would afford.

The contents of the selected nuts in each case were finely mashed and mixed in porcelain mortars. The use of aqueous extracts of weighed samples as reducing agents was found to be unsatisfactory. Ten 2-gm. samples of mash from each of the two lots were quickly but roughly weighed out and placed on individual papers. This permitted duplicate determinations by using five oxidation-reduction indicators. These samples were quickly transferred to test-tubes containing 15 cc. of solution plus an oxidation-reduction indicator.

The solution employed was a modified HOAGLAND's solution (4), in which the phosphate was supplied as the di-potassium hydrogen phosphate instead of the mono-potassium di-hydrogen phosphate, and in which iron and manganese were omitted. Such a solution has a pH of 6.8-7, and is considerably buffered. The temperature of the room in which the determinations were made was close to 30° C. The solution was first poured out into beakers; sufficient indicator was then added to give a deep color; and after being stirred, 15 cc. of the solution was run into each of four test-tubes previously graduated to

15 cc. Another similar set of ungraduated test-tubes, but of uniform diameter and color, were reserved for the final color comparisons.

Of the oxidation-reduction indicators that were tried (6), some were found (table I) to be reduced too easily to be of value in the present tests. The indigo sulphonates were the most satisfactory of the group designated as excellent in table I.

TABLE I  
RELATIVE POSITIONS OF OXIDATION-REDUCTION INDICATORS

| INDICATOR  | POTENTIAL IN<br>VOLTS AT pH 7<br>AND 30° C. (6) |
|--|---|
| Too easily reduced                               |   |
| 2- Chloro-indophenol (o-chlorophenol indophenol) | +0.233  |
| Indophenol (phenol indophenol)                   | +0.228  |
| 2- Methyl-indophenol (o-cresol indophenol)       | +0.195  |
| Excellent  |   |
| 1- Naphthol 2-sulphonate indophenol              | +0.123  |
| Methylene blue                                   | +0.011  |
| Indigo-tetrasulphonate                           | -0.046  |
| Indigo-disulphonate                              | -0.125  |
| Indigo-monosulphonate                            | -0.156  |

The procedure of the tests was as follows. The weighed samples of mashed walnut kernels were in each case rapidly transferred from the papers to the test-tubes containing the measured amount of solution plus indicator, and thoroughly shaken under comparable conditions. The tubes were left standing from 15 to 30 minutes until the reduction was well under way. With little practice one can learn the concentration of indicator required to bring about the most contrast in color at the end of the period of standing. When the tubes had stood the desired period, the comparable tubes were shaken at the same time and at once filtered into the test-tubes already prepared. A good grade of filter paper (Whatman no. 40) was used, since it was found that ordinary paper permits finely divided particles to pass through into the solution, giving an opalescence that interferes considerably with the color comparisons.

A 200 watt 120 volt nitrogen-filled lamp served as a source of light, in front of which was placed a piece of Dalite glass. The color comparisons were made against a porcelain plate background, using reflected light. Although it is desirable to compare the time required



for equal reductions, a comparison of the degree of reduction in equal time appeared to be the most practical method and gave consistent results.

Many electric ovens, especially when in use for some time, give temperatures that may be far from uniform. In preparing heated nuts for experimental purposes, therefore, maximum and minimum thermometers as well as other accurate thermometers were placed in the oven on the same level as the nuts to insure accurate temperature control. In all of the tests the walnut varieties were mixed indiscriminately, so that varietal differences are not of much importance. Taking walnuts heated at 110° F. for 24 hours as a standard, and a sample heated to 120° F. or above, it was found to be a relatively simple matter to determine which of the two had been "overheated." About twenty-five samples of walnuts known to have been overheated during dehydration at dehydration plants or in the laboratory were all found to show a greater degree of indicator reduction in a given time than the control nuts. Moreover these tests were run within a few days after the nuts were dehydrated.<sup>2</sup> When such samples were stored for a month or more, the tests were repeated with the same results but somewhat more pronounced. It was possible with the aid of the adopted standard to detect nuts heated supposedly at 110° F. for 24 hours but during which time the temperature had been at 117°, 126°, or 130° F. respectively for a few hours. Nuts heated at 120° for 18 hours showed greater reducing action than nuts heated at 110° for 24 hours. Twelve-hour heating of nuts at 100°-103° F. gave less reduction than 24-hour heating at the same temperature, and the former showed "overheating" in comparison with a sample of the unheated nuts.

A lot of nuts that had been subject to air-drying in storage for about two months were divided into samples of several hundred nuts each. Of samples heated for 24 hours, those at 120° showed overheating with respect to those heated at 110° F. When samples were heated to 120° for 12 hours they still showed overheating.

With respect to the standard samples heated at 110° F. for 24 hours, heating at 120° for 24 hours showed slightly greater reducing action than heating at 112°-114° for 24 hours. It is possible therefore

<sup>2</sup> The writer appreciates the assistance given in some of the preliminary routine by Mr. O. L. BRAUCHER, Field Investigator of the California Walnut Growers' Association.

to study by such a method the equivalent effects of heating. As the "overheating" becomes excessive the reducing action becomes the more rapid, and when "overheating" is doubtful the differences in color with the several indicators will usually be very small if not indistinguishable or inconsistent. When all of the indicators show a decidedly more rapid reduction in the one case than in the other, it is practically certain that "overheating" has taken place in the former sample.

### Summary

1. A simple and delicate method is described in which oxidation-reduction indicators are used in buffers of approximate neutrality for the detection of "overheating" in walnuts.
2. Mashed walnut kernels show a strong reducing power which is increased when the nuts are "overheated."
3. It is possible by the method described to study equivalent effects of heating when the temperature and time of heating are varied.
4. "Overheating" is quite as satisfactorily detected in freshly dehydrated nuts as in those held for some time in storage.

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### LITERATURE CITED

1. BATCHELOR, L. D., and CHRISTIE, A. W., assisted by GUTHIER, E. H., and LARUE, R. G., Sun-drying and dehydration of walnuts. Univ. Calif. Agric. Exp. Sta. Bull. 376. 1-26. 1924.
2. CLARK, W. M., and others, Studies on oxidation-reduction. U.S. Public Health Reports, Reprints 823, 826, 834, 848, 904, 915, 1001, 1017; Supplements 54, 55, 61, 66.
3. HIBBARD, R. P., and MILLER, E. V., Biochemical studies on seed viability. I. Measurements of conductance and reduction. Plant Physiol. 3:335-352. 1928.
4. HOAGLAND, D. R., Optimum nutrient solutions for plants. Science N.S. 52: 562-564. 1920.
5. NEEDHAM, JOSEPH, and NEEDHAM, DOROTHY M., Hydrogen-ion concentration and the oxidation-reduction potential of the cell-interior: a microchemical study. Proc. Roy. Soc. B. 98:259-286. 1925.
6. TAYLOR, W. A., The A B C of hydrogen-ion control. LaMotte Chemical Products Co. 4th ed., 2d printing. pp. 105-110. 1928.

# CURRENT LITERATURE

## BOOK REVIEWS

### Gnetales

In 1904, soon after his appointment as Bolus Professor of Botany in the South African College at Cape Town, Professor PEARSON visited the Damara-land Desert and made a thorough field study of *Welwitschia*, which at that season was in full bloom. He took photographs and collected material for a morphological investigation. During subsequent trips he traced the change in the flora from Kalahari Plateau into the desert. As he worked upon the morphological material, he realized the need for a comparison with *Gnetum*, and accordingly visited Angola, where he made field studies and collected material. As his papers began to appear, he was prevailed upon to write a general account as a volume in the series of Cambridge Botanical Handbooks. Much of the book was written before he died, in November, 1916. Professor SEWARD took the unfinished manuscript and, with the assistance of Mrs. THODAY, who had written several papers on *Ephedra* and *Welwitschia*, completed the work.<sup>1</sup> The last chapter, in its present form, is based upon PEARSON's notes and upon two papers which were published after his death.

There is a preface by the editor, A. C. SEWARD, and five chapters: (I) Habit, distribution, ecology, taxonomy; (II) Vegetative morphology and anatomy; (III) The inflorescence and flower; (IV) Reproduction; (V) Theoretical chapter. There is also an extensive bibliography and index.

Throughout the book the treatment shows a wide knowledge of Gnetales in the field; and the author's thorough training in taxonomy increased the value of the ecological descriptions.

So few botanists have studied *Welwitschia* in the field that it is worth while to note a few points. Large plants attain, at the top, a diameter of 4 feet, but seldom extend more than 18 inches above the surface. Often they are nearly covered with sand, which frequently gets into the depression in the top, and in moist seasons supports a growth of epiphytic grasses and other plants. Individuals growing close together may fuse into irregular complexes. The young leaves are erect but soon become depressed, so that the distal ends rest upon the ground and become torn to ribbons by the wind. The plant produces only two leaves, which are constantly renewed at the base but which seldom reach more than 6 feet in length because they die off at the distal end. The roots are remarkably long, penetrating to moist soil. The dry habitat may not be necessary,

<sup>1</sup> PEARSON, H. H. W., Gnetales. Large 8vo. pp. vi+194. pls. 3 and portrait. figs. 90. Cambridge University Press. 1929.

or even desirable, for plants grown in the greenhouse thrive better in the orchid room than in the room for desert plants.

The range of *Welwitschia* from north to south is about 700 miles, from the mouth of the San Nicolán River in latitude  $14^{\circ}$  S. to a locality along the Rio Caroca in latitude  $15^{\circ}50'$  S. Plants extend from the sea coast inland for about 20 miles; at Walvis Bay about 30 miles. In parts of this range the average yearly rainfall is only 1 inch. The classic station, where the plant was discovered by WELWITSCH, is near Cape Negro, on a plateau about 400 feet in height. When PEARSON visited this locality in April, 1909, after a period of exceptionally heavy rainfall, a luxuriant growth of *Aristida* made the region look like a corn-field, so that *Welwitschia* was hidden by the tall grass. Seedlings were numerous. It is probable that seeds retain their vitality for several years and produce abundant seedlings in such seasons.

In treating the vegetative anatomy, special attention is given to seedlings, and conditions in the three genera are compared. In discussing the "perianth" of *Welwitschia*, the conclusion is reached that the mere fact that it is vascularized is not sufficient to establish the claim that it is a foliar structure: just what it may be is still to be proved. If the expanded inner integument of the male flower represents an abortive stigma, why is it retained in the male flower, where it does not function, but lost in the female flower, where it might have functioned? Although the other parts of the flower are so diverse in the three genera, the ovules are very similar. A series of diagrams emphasizes the similarities.

The reduction in the female gametophytes is traced, and the three genera are compared, the series being *Ephedra*, *Welwitschia*, *Gnetum*, with *Gnetum* as the most reduced form, having nearly reached the angiosperm condition. So far as the extent of development is concerned, the female gamete in *Welwitschia* is formed in the twelfth or thirteenth division from the megaspore mother cell; in *Gnetum*, somewhat earlier; and in angiosperms, still earlier. Fusions of nuclei in the female gametophyte, stimulating the development of endosperm, are compared with the triple fusion in the double fertilization of angiosperms.

The interrelationships of the three genera are still obscure, and there is no proof of any near affinity; but there is reason to believe that they are the existing remnants of an ancient race which was once more numerous represented. Perhaps the ancestors of the angiosperms were not far removed from *Gnetum*. In anatomy, and in the female gametophyte, the resemblance is to conifers.

The dichasial cymes of Gnetales are not found in recent gymnosperms, but they occur in *Wielandiella*. The resemblance between the long micropylar tubes of *Gnetum* and the Bennettitales, and the shape of the integuments have been emphasized by THODAY and BERRIDGE; but these comparisons afford no proof of phylogenetic relationship.

The general conclusion is that relationships of the three genera of the Gnetales are as obscure now as they have been at any time within the past decade; but the evidence indicates that, in spite of the occurrence in them of some angiosperm characters, they are essentially gymnosperms.—C. J. CHAMBERLAIN.

### Belgian coal ball studies

Miss LECLERCQ has continued her initial studies of Belgian coal balls<sup>2</sup> by a series of equally interesting and valuable notes.<sup>3</sup> The text in all these publications is supplemented by excellent illustrations, which are based upon photographs accompanied by ample legends.

It is a rich flora which is presented. It belongs to the Lower Carboniferous and to the Lower and Middle sections of the Upper Carboniferous. These last two sections of the Upper Carboniferous should be numbered in accordance with the recently established classification of the European coal measures, Westphalian A and B, and correspond to our Pottsville.

The following species are described: *Calamites communis* Binn., *C. binneyana* Schim., *C. casheana* Will., *C. sp.*, *Sphenophyllum plurifoliatum* Will., *S. gilkineti* Suz. Leclercq., *S. dawsoni* Will., *Lepidodendron selaginoides* Carr., *L. harcourtii* With., *Lepidophloios fuliginosus* Will., *Lepidostrobus fuliginosus* Will., *Lepidocarpon* sp., *Bothrodendron mundum* Will., *Sigillaria* sp., *Stigmaria ficoides* Sternb., *S. lohesti* Suz. Leclercq., *S. bacupensis* Will., *S. weissiana* Leclercq., *Etapteris scottii* P. Bertrand, *E. lacatei* Ren., *Ankropteris bibractensis* var. *westphaliensis* P. Bertrand, *A. corrugata (stipe)* Will., *Stauropteris oldhamia* Binn., *Botryopteris ramosa* Will., *B. cylindrica* Will., *Psaronius* sp., *Lyginopteris* sp., *Cordaites felicitis* Benson, *C. weristeri alpha* Leclercq., *C. weristeri beta* Leclercq., *Amyelon radicans* Will., *Mesoxylon* sp., *Trigonocarpus* sp.

The Belgian coal balls offer a rich field for the study of the structure of carboniferous plants, and it is to be hoped that Miss LECLERCQ will continue to publish contributions from this source.—A. C. NOÉ.

### Protoplasm

An international series of monographs<sup>4</sup> on protoplasm has recently been started under the editorship of F. WEBER (Graz) and L. V. HEILBRUNN (Woods Hole), with the collaboration of R. CHAMBERS (New York), E. FAURÉ-FREMIET (Paris), H. FREUNDLICH (Berlin), E. KÜSTER (Giessen), F. E. LLOYD (Montreal), H. SCHADE (Kiel), W. SEIFRIZ (Philadelphia), J. SPEK (Heidelberg), and W. STILES (Reading).

Volume III in this series deals with the pathology of the plant cell,<sup>5</sup> and its first part is devoted to the pathology of protoplasm, for which it intends to be an introduction rather than a monograph.

<sup>2</sup> LECLERCQ, SUZANNA, Introduction à l'étude anatomique des végétaux houillers de Belgique: Les Coal balls de la couche Buxharmont des Charbonnages de Wéristér. 4vo. pp. 79. pls. 49. Mém. Soc. Géol. Belgique. Liège. 1925.

<sup>3</sup> ———, Les Végétaux à structure conservée du houiller Belge. Note 1. 8vo. pp. 14. figs. 6. Note 2. pp. 7. figs. 6. Note 3. pp. 14. figs. 7. Note 4. pp. 7. Ann. Soc. Géol. Belgique t. LI. Bulletin. Liège. 1928.

<sup>4</sup> Protoplasma-Monographien. Berlin: Gebrüder Borntraeger. 1928.

<sup>5</sup> KÜSTER, E., Pathologie der Pflanzenzelle. Teil I: Pathologie des Protoplasmas. pp. viii+200. figs. 36. Berlin: Gebrüder Borntraeger. 1929.

The book is divided into two chapters: changes of form (Formwechsel), and structural changes (Strukturwechsel). The first chapter is subdivided into the following sections: (1) plasmolysis; (2) artificial deformation; (3) division protoplasts; (4) dislocation of protoplasm; (5) protomoptysis and related phenomena; (6) local necrosis; and (7) increase in volume of denuded protoplasts by swelling.

The second chapter contains such sections as (1) changes in the stratification of protoplasm; (2) coagulation of protoplasm; (3) degeneration through vacuoles or bubbles; (4) swelling of protoplasm.

The appendix deals with (1) the change in protoplasmal structure produced by stains and other chemicals; (2) the precipitation of tannin and other precipitates in the cell sap; and (3) the importation of foreign substances into the living protoplasts. Constant references to the very extensive bibliography are an addition to the usefulness of the book. It would undoubtedly gain in value, especially for the beginner, if it were more amply illustrated.—A. C. NOÉ.

### Mushrooms and toadstools

A recent volume by GÜSSOW and ODELL<sup>6</sup> will enable the collector and student of fleshy fungi to add considerably to his understanding of the fungi which he is likely to meet in Canada, and to some extent, in the northern United States. The volume abounds in excellent plates, which are supplemented by brief descriptions. The introductory chapters include a discussion of the nature, structure, and classification of fungi, and a guide to the genera discussed.—G. K. K. LINK.

### Fungi of Middle Europe

Numbers 5, 6, 7, and 8 of volume I of *Die Pilze Mitteleuropas*<sup>7</sup> have appeared, and continue the excellence of the earlier numbers. Number 5 carries two colored plates devoted to *Boletus regius*, *B. appendiculatus*, and *B. aestivalis*, as well as a plate in black and white devoted to other *Boleti*. The text is devoted to a discussion of *B. regius*. Number 6 carries two colored plates and text devoted to *B. luridus*. Number 7 carries two colored plates, one devoted to *B. elegans* and the other to *B. viscidus*, and a plate in black and white devoted to various *Boleti*. The text is devoted to *B. flavus* and *B. viscidus*. Number 8 carries two colored plates and text devoted to *B. luteus* and *B. variegatus*. It is unfortunate that a work of similar nature is not under way for North American fungi.—G. K. K. LINK.

<sup>6</sup> GÜSSOW, H. T., and ODELL, W. S., *Mushrooms and toadstools*. Div. Botany, Dominion Experimental Farms. pp. 274. pls. 128. Ottawa. 1927.

<sup>7</sup> *Die Pilze Mitteleuropas*, under the editorship of KNEP, H. (Berlin), CLAUSSEN P. (Marburg), and BASZ, J. (Stuttgart). Leipzig: W. Klinghardt.

# THE BOTANICAL GAZETTE

May 1930

## IRON-ION CONCENTRATION IN RELATION TO GROWTH AND OTHER BIOLOGICAL PROCESSES

E. F. HOPKINS

(WITH NINE FIGURES)

### Introduction

In a paper by HOPKINS and WANN (6) it was shown that with a given amount of iron in the culture solution, the growth of *Chlorella* sp. varied with the concentration of sodium citrate. The higher the amount of citrate the less the growth. As an explanation of this fact the suggestion was made that increasing the citrate content depressed the iron-ion concentration through the formation of a complex iron compound which ionizes in such a manner as to give only a very small concentration of iron ions. This idea was based on the work of USPENSKI (9, 10, 11), who summarized his investigations in a recent paper (10). According to him not only can the depression of growth be explained in this way, but also the prevention of the toxicity of iron. Large amounts of iron which in certain cases would be very toxic, in the presence of organic matter may be entirely harmless. In the study just mentioned (6), it was found in one case that with a small amount of sodium citrate (0.04 gm. per culture) only 0.0075 mg. of iron was necessary to give some growth, while with a large amount (0.15 gm. per culture) 0.075 mg. of iron, or ten times as much, is required.

The present work was undertaken to carry out experiments with a greater range of iron and citrate concentration to determine the

effect of these various solutions on growth; also if possible to discover the relation of the concentration of iron ions to the development of *Chlorella* sp.

### Effect of iron ions on growth

EXPERIMENT 1: VARIATION IN CITRATE CONTENT WITH CONSTANT AMOUNT OF IRON.—This experiment was designed to determine the effect of varying the amount of sodium citrate while keeping the iron constant. Practically the same experimental procedure as described by HOPKINS and WANN (6) was used in this case. The stock solutions were made up in two parts as follows:

| SOLUTION A                                | GM.  | SOLUTION B                            | GM.   |
|---|------|---------------------------------------|-------|
| NH <sub>4</sub> NO <sub>3</sub> .....     | 2    | K <sub>2</sub> HPO <sub>4</sub> ..... | 23.23 |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O..... | 0.8  | H <sub>2</sub> O.....                 | 1000  |
| Glucose.....                              | 40.0 |                                       |       |
| H <sub>2</sub> O.....                     | 1000 |                                       |       |

These were then mixed as shown in table I, to make the individual culture solutions. As 0.1 mg. Fe per culture was desired in each case, 0.07 mg. of iron was added, since it was estimated that about 0.03

TABLE I  
COMPOSITION OF CULTURE SOLUTION IN EXPERIMENT 1

| CULTURE | SODIUM CITRATE |                            | IRON |                           | H <sub>2</sub> O TO<br>MAKE 12.5<br>(CC.) | SOLUTION A<br>(CC.) | SOLUTION<br>B†<br>(CC.) |
|---------|----------------|----------------------------|------|---------------------------|---|---------------------|-------------------------|
|         | Gm.            | Cc. 4 per<br>cent solution | Mg.  | Cc. standard<br>solution* |   |                     |                         |
| 1.....  | 0.01           | 0.25                       | 0.07 | 2.0                       | 10.3                                      | 12.5                | 25                      |
| 2.....  | 0.02           | 0.50                       | 0.07 | 2.0                       | 10.0                                      | 12.5                | 25                      |
| 3.....  | 0.04           | 1.00                       | 0.07 | 2.0                       | 9.5                                       | 12.5                | 25                      |
| 4.....  | 0.06           | 1.50                       | 0.07 | 2.0                       | 9.0                                       | 12.5                | 25                      |
| 5.....  | 0.08           | 2.00                       | 0.07 | 2.0                       | 8.5                                       | 12.5                | 25                      |
| 6.....  | 0.10           | 2.50                       | 0.07 | 2.0                       | 8.0                                       | 12.5                | 25                      |
| 7.....  | 0.15           | 3.75                       | 0.07 | 2.0                       | 6.8                                       | 12.5                | 25                      |
| 8.....  | 0.20           | 5.00                       | 0.07 | 2.0                       | 5.5                                       | 12.5                | 25                      |

\* Prepared by diluting 7 cc. of an iron solution containing 0.5 mg. Fe per cc. to 100 cc.

† Sterilized separately.

mg. would be derived from impurities. The two components of each culture solution were sterilized separately as indicated in the table and mixed when cool. It is evident, then, that in the final culture solution, A was diluted to four times its original volume and B twice.



Each solution was prepared in quadruplicate, three of the flasks being inoculated with a suspension of *Chlorella* cells while the fourth was retained for initial pH and iron determinations.

TABLE II  
RESULTS OBTAINED IN EXPERIMENT I; TIME TWO WEEKS

| SOLUTION | CITRATE PER CULTURE (GM.) | PH OF SOLUTION  |     |     |      | IRON (MG.)      |         | DRY WEIGHT OF CROP (MG.) |       |       | AVERAGE CROP (MG.) |
|----------|---------------------------|-----------------|-----|-----|------|-----------------|---------|--------------------------|-------|-------|--------------------|
|          |                           | Initial control | A   | B   | C    | Initial control | Final A | A                        | B     | C     |                    |
| 1.....   | 0.01                      | 7.4             | 7.2 | 7.2 | 7.2  | 0.081           | 0.081   | 148.3                    | 157.3 | 134.1 | 146.6              |
| 2.....   | 0.02                      | 7.4             | 7.2 | 7.2 | 7.2  | 0.093           | 0.082   | 163.5                    | ..... | 136.3 | 149.9              |
| 3.....   | 0.04                      | 7.4             | 7.2 | 7.2 | 7.2  | 0.089           | 0.082   | 183.8                    | 169.0 | 163.0 | 171.9              |
| 4.....   | 0.06                      | 7.4             | 7.2 | 7.2 | 7.2  | 0.098           | 0.088   | 158.0                    | 163.5 | 156.8 | 159.4              |
| 5.....   | 0.08                      | 7.4             | 7.2 | 7.2 | 7.2  | 0.096           | 0.087   | 145.3                    | 154.2 | 155.3 | 151.6              |
| 6.....   | 0.10                      | 7.4             | 7.2 | 7.2 | 7.2  | 0.094           | 0.090   | 120.9                    | 131.6 | 149.0 | 133.8              |
| 7.....   | 0.15                      | 7.4             | 7.4 | 7.2 | 6.85 | 0.094           | 0.096   | 54.4                     | ..... | ..... | 54.4               |
| 8.....   | 0.20                      | 7.3             | 7.5 | 7.6 | 7.5  | 0.096           | 0.102   | 7.4                      | 7.2   | 16.8  | 10.5               |

Inoculations were made February 19, 1927, and by February 23 growth was evident in certain cultures. The amount of growth appeared to decrease from cultures 1 to 8. One week after inoculation very good growth was noted in cultures 1, 2, 3, and 4; beginning with culture 5 there was less growth, while in 7 there was only slight growth and still less in 8. At the end of two weeks the dry weights were determined. These results are shown in table II and plotted graphically in fig. 1.

The data show practically no change in pH nor in the total iron concentration, but a marked variation in the amount of growth. The latter rises to a maximum at a citrate concentration of 0.04 gm. per culture, and then

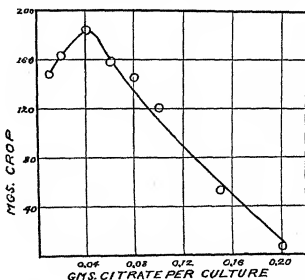


FIG. 1.—Effect of citrate on growth of *Chlorella* in solutions having constant amount of total iron (experiment 1).

rapidly drops, until at 0.20 gm. it is very little. Undoubtedly a further increase in the citrate would have inhibited growth entirely. This is a striking confirmation of the idea previously expressed that citrate does have an important influence on the growth of *Chlorella*, for in this experiment the total soluble iron was the same in all cultures and the pH remained practically constant.

EXPERIMENT 1A: EFFECT OF CITRATE CONCENTRATION ON REACTION FOR FERRIC IRON.—To obtain a relative measure of the concentration of iron ions present in the culture solutions used in experiment 1, the following procedure, based on the method of USPENSKI, was carried out. To 10 cc. of each of the control solutions (uninocu-

TABLE III

| CULTURE SOLUTION NUMBER | GRAMS CITRATE<br>PER CULTURE | PERCENTAGE<br>Fe (SCN) <sub>3</sub> SOLU-<br>TION NO. 1 = 100 |
|-------------------------|------------------------------|---|
| 1.....                  | 0.01                         | 100.0   |
| 2.....                  | 0.02                         | 55.1  |
| 3.....                  | 0.04                         | 44.1  |
| 4.....                  | 0.06                         | 42.2  |
| 5.....                  | 0.08                         | 31.5  |
| 6.....                  | 0.10                         | 33.7  |
| 7.....                  | 0.15                         | 21.2  |
| 8.....                  | 0.20                         | 16.7  |

lated) from experiment 1 was added 2 cc. of N/2 HCl. This adjusted the solutions to an acid reaction at which the  $\text{Fe}(\text{SCN})_3$  color could be observed. Ten cc. of 10 per cent KSCN and 1 cc. of a solution of potassium persulphate (5 mg. per cc.) were then added to each of these mixtures and the volume made up to 25 cc. in a 50 cc. graduated cylinder. Ether was added to each cylinder up to the 40 cc. mark, and the  $\text{Fe}(\text{SCN})_3$  transferred to this ether layer. The relative intensity of color in each ether layer was determined in a colorimeter and the results expressed as percentages of the color developed in solution no. 1. The results obtained are shown in table III.

The data shown in table III and fig. 1a show that as the citrate content increases the ferric-ion concentration decreases, in a manner which corresponds very well with the falling off in growth determined in experiment 1. These results indicate that the effect of the citrate on growth is due to its depressing action on the iron-ion con-

centration, which is the physiologically important factor involved, rather than the total iron in the solution.

EXPERIMENT 2: VARIATION IN TOTAL IRON CONTENT WITH CONSTANT AMOUNT OF CITRATE.—In this case a rather large and constant amount of sodium citrate was used and the concentration of iron

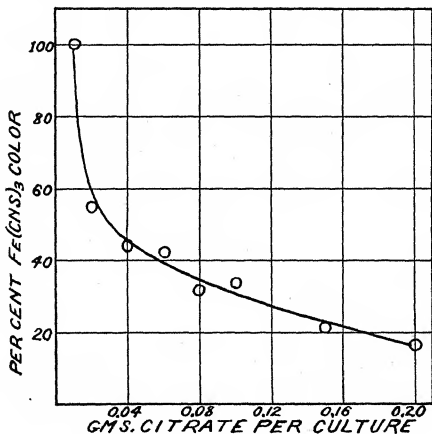


FIG. 1a.—Effect of citrate on reaction for ferric ions in solutions used in experiment 1; solution no. 1=100 per cent (experiment 1a).

was varied. The same stock solutions and technique were used as in experiment 1. The compositions of the solution are shown in table IV.

The summarized data for the experiment are given in table V, and also presented graphically in fig. 2. In the cultures with high iron content the amount of sodium citrate (0.15 gm. per culture) was not sufficient in all cases to keep this amount of iron in solution, and some precipitation occurred. This precipitation took place progressively from series A, which was on the side of the shelf away from the window, to series C which was toward the window. For instance,

in series *A* an iron precipitate was observed in cultures 8 and 9; in *B* in cultures 7, 8, and 9; and in *C* in cultures 6, 7, 8, and 9. It is suggested that increasing illumination in those cultures nearest the

TABLE IV  
COMPOSITIONS OF SOLUTIONS USED IN EXPERIMENT 2  
WITH INITIAL IRON AND PH VALUES

| CULTURE | SODIUM CITRATE (GM.) | IRON     |       | PH INITIAL CONTROL |
|---------|----------------------|----------|-------|--------------------|
|         |                      | Expected | Found |                    |
| 1.....  | 0.15                 | 0.05     | 0.067 | 7.4                |
| 2.....  | 0.15                 | 0.07     | 0.074 | 7.4                |
| 3.....  | 0.15                 | 0.10     | 0.144 | 7.4                |
| 4.....  | 0.15                 | 0.50     | 0.81  | 7.3                |
| 5.....  | 0.15                 | 1.00     | 1.64  | 7.2                |
| 6.....  | 0.15                 | 2.00     | 2.50  | 7.1                |
| 7.....  | 0.15                 | 3.00     | 3.52  | 6.8                |
| 8.....  | 0.15                 | 4.00     | 4.70  | 6.7                |
| 9.....  | 0.15                 | 5.00     | 6.00  | 6.6*               |

\* Increase in H-ion concentration due to acid added in standard iron solution.

TABLE V  
RESULTS OBTAINED IN EXPERIMENT 2; TIME TWO WEEKS  
SODIUM CITRATE 0.15 GM. PER CULTURE

| SOLUTION NO. | IRON (MG.) |                 |                | PH OF SOLUTIONS |       |      |      | DRY WEIGHT OF CROP (MG.) |       |       |         |
|--------------|------------|-----------------|----------------|-----------------|-------|------|------|--------------------------|-------|-------|---------|
|              | Added      | Initial control | Final series A | Initial control | Final |      |      | A                        | B     | C     | Average |
|              |            |                 |                |                 | A     | B    | C    |                          |       |       |         |
| 1.....       | 0.05       | 0.067           | 0.060          | 7.40            | 7.6   | 7.6  | 7.6  | 1.7                      | 1.7   | 1.1   | 1.5     |
| 2.....       | 0.07       | 0.074           | 0.075          | 7.40            | 7.6   | 7.6  | 7.6  | 8.0                      | 13.5  | 20.3  | 13.9    |
| 3.....       | 0.10       | 0.144           | 0.108          | 7.40            | 7.5   | 7.4  | 7.5  | 35.8                     | 48.5  | 38.2  | 40.8    |
| 4.....       | 0.50       | 0.810           | 0.555          | 7.30            | 7.2   | 7.2  | 7.3  | 145.6                    | 146.9 | 125.4 | 139.3   |
| 5.....       | 1.00       | 1.640           | 1.170          | 7.20            | 7.2   | 7.2  | 7.2  | 152.9                    | 148.6 | 125.6 | 142.3   |
| 6.....       | 2.00       | 2.500           | 2.300          | 7.10            | 7.2   | 7.2  | 7.2  | 156.7                    | 152.1 | 109.2 | 139.0   |
| 7.....       | 3.00       | 3.520           | 3.720          | 6.85            | 6.95  | 7.1  | 7.1  | 134.6                    | 126.5 | ..... | 130.5   |
| 8.....       | 4.00       | 4.700           | 0.148          | 6.75            | 6.85  | 6.85 | 6.85 | 137.5                    | 111.0 | ..... | 124.2   |
| 9.....       | 5.00       | 6.000           | 0.005          | 6.60            | 6.75  | 6.75 | 6.75 | 122.0                    | 118.9 | ..... | 124.4   |

window may offer an explanation, light accelerating certain reactions which result finally in precipitation of the iron. This is borne out by the fact that in the control series, which was left on a laboratory table under much weaker illumination, there was no precipitation after a month's time except in the case of solution 9. It is of interest

to note that BAUDISCH and WELO (3) have shown that precipitation of iron from certain complex iron compounds present in natural mineral waters is accelerated by light. This loss of soluble iron is reflected in the growth (table IV). Taking this into account, however, the result is what should be expected from our theory. Comparison

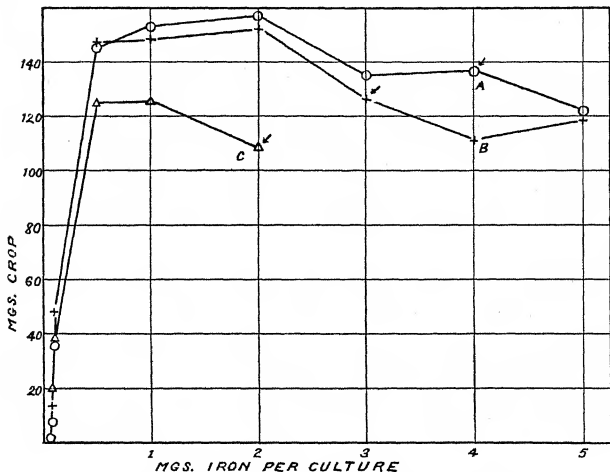


FIG. 2.—Effect of iron on growth of *Chlorella* in solutions having constant amount of citrate; arrows indicate concentrations of iron at which precipitation began to take place (experiment 2).

with experiment 1 can conveniently begin with cultures no. 3. These cultures, which contain 0.1 mg. of iron and 0.15 gm. of sodium citrate, correspond to cultures no. 7 in experiment 1, which have the same iron and citrate content. The growth is approximately the same. In experiment 2, when the iron content is decreased the growth falls off, until at the lowest iron content there is practically none because the iron-ion concentration has become very small. On

the other hand, the iron-ion concentration can be increased above that in cultures no. 3 by increasing the total iron content above 0.1 mg. per culture. When this is done, as in cultures 4-9, there are marked increases in growth until the precipitation just described takes place.

This appears to rule out the possibility that in experiment 1 the depression of growth with increasing concentration of citrate is due to the toxicity of the latter, because growth depressed by increasing the citrate content may be increased by further addition of iron.

**EXPERIMENT 3: VARIATION IN CITRATE AND IRON CONCENTRATIONS.**—The first two experiments suggested that it might be desirable to determine the most favorable ratio between iron and sodium citrate for growth. The sodium citrate and the iron were varied so that every citrate concentration had seven different concentrations of iron, and vice versa. This was carried out as follows. The stock solutions were as given in experiment 1, except that A was only one-half as concentrated. In the final culture solutions, however, the concentration was the same since only one dilution was made.

Both A and B before mixing were treated with  $\text{Ca}_3(\text{PO}_4)_2$  and then filtered, to remove the iron impurities by adsorption. After addition of the citrate and iron the solutions were sterilized separately in 25 cc. portions and mixed when cool as before. The cultures were inoculated April 9 with *Chlorella* cells, but by April 13 there was practically no growth in any of the cultures, although some showed a trace of colorless cells. One culture in a Jena flask (used accidentally) showed a slight greenish growth. Growth continued to be slow, and final notes were taken April 20, eleven days after inoculation. It was decided that the crops were too small for dry weight determinations and these were not made. In table VI the composition of the different solutions is shown and the results of the observations recorded. Those solutions in which precipitation of iron occurred are marked "ppt." In this table three plus signs represent what was judged to be the best growth in each numerical series. The results are interesting in that they show with increasing iron a shifting of the maximum

growth points to higher citrate concentrations. By approximate interpolation we obtain the following:

|  |       |       |      |      |      |      |
|--|-------|-------|------|------|------|------|
| Sodium citrate (gm.<br>per culture)..... | 0.01  | 0.02  | 0.03 | 0.04 | 0.06 | 0.10 |
| Iron mg. at maxi-<br>mum growth.....     | 0.015 | 0.075 | 0.20 | 0.40 | 0.50 | 1.00 |

It is also of interest to note the concentration of iron at which growth is inhibited by various amounts of citrate, which are estimated from table VI to be:

| GM. CITRATE PER<br>CULTURE | IRON CONCENTRATION<br>AT WHICH GROWTH<br>IS INHIBITED |
|----------------------------|---|
| 0.050                      | 0.01  |
| 0.070                      | 0.02  |
| 0.125                      | 0.05  |
| 0.160                      | 0.10  |

The first values might be taken to represent the most favorable concentration of iron ions, or possibly the most favorable ratio be-

TABLE VI  
RESULTS OBTAINED IN EXPERIMENT 3

| IRON (MG.) | SODIUM<br>CITRATE<br>(GM.) | A          | B          | C    | D    | E    | F    | G     |
|------------|----------------------------|------------|------------|------|------|------|------|-------|
|            |                            | 0.01       | 0.02       | 0.03 | 0.04 | 0.06 | 0.10 | 0.15* |
| 1.....     | 0.01                       | +++        | ++         | ++   | +    | -    | -    | -     |
| 2.....     | 0.02                       | +++        | ++         | ++   | ++   | ++   | -    | -     |
| 3.....     | 0.05                       | ++<br>ppt. | +++        | ++   | ++   | ++   | ++   | -     |
| 4.....     | 0.10                       | ++<br>ppt. | +++        | ++   | ++   | ++   | ++   | +     |
| 5.....     | 0.30                       | ++<br>ppt. | +          | ++   | +++  | ++   | o*   | ++    |
| 6.....     | 0.50                       | ++<br>ppt. | +          | ++   | +++  | ++   | ++   | ++    |
| 7.....     | 1.00                       | ++<br>ppt. | ++<br>ppt. | ++   | ++   | ++   | +++  | +     |

\* Contaminated with bacteria.

tween  $[\text{Fe}++]$  and  $[\text{Fe}+++]$ . This will be discussed later. The second series of values in a like manner are those at which the iron-ion concentration is depressed to the point where it is insufficient for growth.

The very poor growth in experiment 3 as a whole was so striking as to demand investigation. It will be recalled that before making up the individual cultures, the stock solutions *A* and *B* had been treated in order to remove any iron impurities; therefore with the results obtained in solutions with entirely sufficient iron present, it seems logical to assume that some other essential element was removed by adsorption during the procedure. To test the effect of the treatment the following experiment was carried out.

EXPERIMENT 3A.—Stock solutions of *A* and *B* were prepared as before and individual culture solutions were made as follows:

I. Five lots of 25 cc. each of *A* without previous adsorption were distributed into flasks.

Five lots of 25 cc. each of *B* also without previous adsorption were distributed into another set of flasks.

To two lots of solution *A* were added 0.07 mg. of iron.

II. To the remainder of solutions *A* and *B* were added separately 5 gm. of  $\text{Ca}_3(\text{PO}_4)_2$ , the flasks being well shaken and allowed to stand over night. The next morning they were filtered and

Five lots of 25 cc. of solution *A* which had been thus treated were distributed into flasks.

Five lots of 25 cc. of solution *B* which had been thus treated were distributed into flasks.

To two lots of solution *A* in this case were also added 0.07 mg. of iron. All the flasks of both I and II were then sterilized, and when cool, solutions *A* and *B* were mixed in each case as usual. They were inoculated April 14 with *Chlorella* cells.

By April 20 the cultures in series I showed excellent growth, those with the added iron showing about twice as much growth as those which contained only the iron due to impurities. In series II there was no growth except in one culture and this was very slight. At the end of four weeks after inoculation series II still showed no weighable amount of growth, while in series I with no added iron there was obtained a growth of 215 mg. from one culture, and with added iron one culture showed a growth of 248 mg. This is strong indication that the treatment with  $\text{Ca}_3(\text{PO}_4)_2$  was responsible for the poor growth in experiment 3, for without the treatment excellent growth was obtained.



Since it was thought that possibly manganese might be the element removed, tests for manganese were made on the glucose (after ashing) and on a solution containing  $\text{MgSO}_4$ ,  $\text{NH}_4\text{NO}_3$ , and  $\text{K}_2\text{HPO}_4$  from the chemicals used in this experiment. A strong test for manganese was obtained in each case, indicating that this may be a factor. In a later experiment (the results of which are not recorded) in which exceptionally pure chemicals were used, very poor growth occurred. This indicates that some impurity is lacking in this case also which is present in the previous solutions. What this is, is not certain and experiments designed toward determining it will be necessary.

TABLE VII  
GROWTH AT END OF ONE WEEK, EXPERIMENT 4

| IRON (MG.) | SODIUM CITRATE (GM.) | A    | B    | C    | D    | E   | F    | G   | H    | I   |
|------------|----------------------|------|------|------|------|-----|------|-----|------|-----|
|            |                      | 0.01 | 0.02 | 0.03 | 0.05 | 0.1 | 0.15 | 0.2 | 0.25 | 0.3 |
| 1.....     | 0.01                 | +    | +    | +    | o    | o   | o    | o   | o    | o   |
| 2.....     | 0.02                 | +    | +    | +    | sl.  | o   | o    | o   | o    | o   |
| 3.....     | 0.05                 | +    | +    | +    | +    | sl. | o    | o   | o    | o   |
| 4.....     | 0.10                 | +    | +    | +    | +    | +   | sl.  | o   | o    | o   |
| 5.....     | 0.30                 | +    | +    | +    | +    | +   | +    | +   | sl.  | o   |
| 6.....     | 0.50                 | +    | +    | +    | +    | +   | +    | +   | +    | sl. |
| 7.....     | 1.00                 | +    | +    | +    | +    | +   | +    | +   | +    | +   |

EXPERIMENT 4: VARIATION IN CITRATE AND IRON CONCENTRATIONS.—A preliminary experiment showed that most of the iron impurity could be removed from the glucose by adsorption with charcoal, and it was decided to repeat experiment 3 exactly as before but first carrying out such an adsorption. The glucose was dissolved in one-half the water to be used in making solution A, animal charcoal was then added, the mixture shaken and allowed to stand over night. It was then filtered and added to an equal volume of water containing in solution the other components of solution A. From then on the procedure was the same as used in experiment 3. In this case, however, two higher citrate concentrations were used in each iron series.

Notes on growth one week after inoculation are shown in table VII. It will be seen from this table that as the citrate is increased a concentration is reached at which growth is inhibited. The concen-

tration of citrate at this point, which might for convenience be designated the "growth extinction point," is different for each iron concentration, becoming greater as the amount increases. Conversely, for any given citrate concentration the amount of iron may decrease until the growth extinction point is reached. As is shown by the table, this variation takes place in an orderly fashion. This will be discussed later.

The dry weights of the crops from these cultures at the end of two weeks are given in table VIII. Because of insufficient citrate some

TABLE VIII  
DRY WEIGHTS OF CROPS IN MG. OBTAINED AFTER TWO  
WEEKS' GROWTH, EXPERIMENT 4

| IRON (MG) | SODIUM CITRATE (GM.) | A     | B     | C    | D    | E    | F    | G    | H    | I    |
|-----------|----------------------|-------|-------|------|------|------|------|------|------|------|
|           |                      | 0.01  | 0.02  | 0.03 | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 | 0.30 |
| 1.....    | 0.01                 | 49.3  | 48.3  | 39.2 | 1.8  | 0.7  | —†   | —    | —    | —    |
| 2.....    | 0.02                 | 44.7  | 44.7  | 25.4 | 14.6 | 0.6  | —    | —    | —    | —    |
| 3.....    | 0.05                 | 55.6  | 36.9  | 47.7 | 48.1 | 11.1 | 1.1  | 3.3  | —    | —    |
| 4.....    | 0.10                 | 49.6  | 62.4  | 54.8 | 45.3 | 32.7 | 13.2 | 1.7  | 0.8  | —    |
| 5.....    | 0.30                 | 43.9* | 61.2  | 54.3 | 50.2 | 57.4 | 39.5 | 26.3 | 18.9 | 4.3  |
| 6.....    | 0.50                 | 43.4* | 40.0* | 56.8 | 56.9 | 59.1 | 56.9 | 45.0 | 29.7 | 18.7 |
| 7.....    | 1.00                 | 61.0* | 42.2* | 66.7 | 59.2 | 68.0 | 72.7 | 68.1 | 49.2 | 33.4 |

\* Some precipitation of iron occurred.

† (—) indicates lack of a weighable amount of cells.

precipitation of iron occurred during the course of the experiment in the following cultures: A5, A6, A7, B6, and B7; therefore the crops in these cases cannot be considered with the others in which this did not occur, since the iron content would be variable during the growth. Final pH values, although determined, are not given in the table. They were practically all pH 7. As would be expected from previous observations, the dry weights became less in each series as the amount of sodium citrate increased, until no weighable amount was present. The results are plotted in fig. 3. In general curves pass through maximum values which are farther toward the right as the iron content increases. This is what we should expect if the iron-ion concentration is the controlling factor, for with higher amounts of iron more citrate is necessary to depress the iron ion to its optimum value. Then with further increase of the citrate, the iron-ion con-

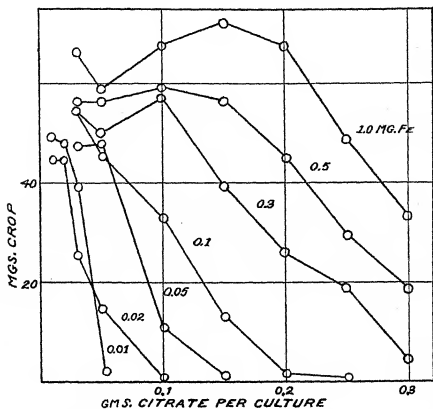


FIG. 3.—Effect of citrate on growth of *Chlorella* at various total iron concentrations (experiment 4).

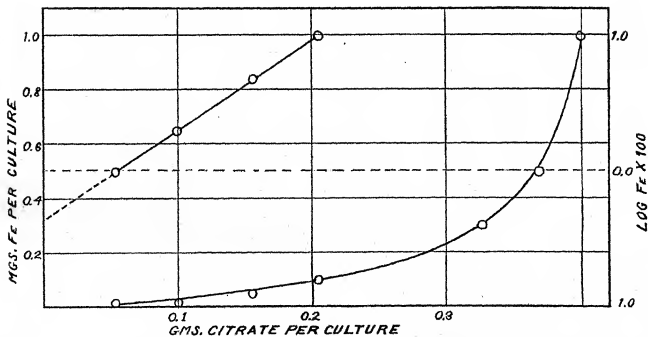


FIG. 4.—Relation between citrate and iron at extinction points for growth, see text for discussion (experiment 4).

centration is depressed below the optimum, so that growth is decreased until finally there are not enough iron ions for growth to be possible. Again in this case the growth extinction points will be farther to the right as the total iron content increases. Based on the hypothesis that the iron-ion concentration is the factor concerned, its value should be the same at all these points. If the total iron values at the growth extinction points are plotted against the citrate contents, the smooth curve shown in fig. 4 is obtained, while if log of total Fe is plotted against citrate the result is a straight line. This line can easily be extrapolated to zero citrate concentration and log total iron, and hence total iron at that point obtained. Since there is no citrate present and therefore no complex iron compound, we can assume complete dissociation. The total iron is then equal to the iron ion. The calculation is as follows:

|   |                         |
|---|-------------------------|
| log Fe per 50 cc. culture solution (by graphic extrapolation) when both characteristic and mantissa are negative..... | = -2.36                 |
| log Fe per 50 cc. culture solution.....   | = 7.64 - 10             |
| Mg. Fe = antilog 7.64 - 10.....   | = 0.00463               |
| Gm. Fe per liter.....   | = 0.000826              |
| Fe-ion concentration $\frac{0.000826}{56}$ .....  | = $1.47 \times 10^{-6}$ |

A Fe-ion concentration of  $1.47 \times 10^{-6}$  as thus obtained is therefore an approximate and provisional value, representing the minimum for growth, until further experiments make it possible to check with other direct or indirect determinations. As was to be expected the value is a low one.

EXPERIMENT 5: EFFECT OF VARIATION IN CITRATE AND IRON CONTENTS ON GROWTH OF BACTERIA.—When the cultures in experiment 4 were filtered to obtain the crops of *Chlorella*, the filtrates became contaminated, principally with motile rod-shaped bacteria. The development of these bacteria in the solutions took place in a manner so similar to *Chlorella* that observations were made as to the effect of citrate in depressing their growth. As with *Chlorella*, the greater the amount of total iron the greater the amount of citrate necessary to inhibit growth. These observations are recorded in table IX. The result is practically the same as that shown in table VII for *Chlorella*.

In order to obtain an idea as to the relative numbers of bacteria in the cultures, nephelometric determinations were made. Culture A1 was taken as 100 and used as a standard with which to compare the others. The results are given in table X.

TABLE IX  
OBSERVATION IN GROWTH OF BACTERIA, EXPERIMENT 5

| IRON (MG.) | SODIUM CITRATE (GM.) | A    | B     | C    | D    | E   | F    | G   | H    | I   |
|------------|----------------------|------|-------|------|------|-----|------|-----|------|-----|
|            |                      | 0.01 | 0.02  | 0.03 | 0.05 | 0.1 | 0.15 | 0.2 | 0.25 | 0.3 |
| 1.....     | 0.01                 | ++   | +     | +    | sl.  | o   | o    | o   | o    | o   |
| 2.....     | 0.02                 | +    | ..... | +    | +    | o   | o    | o   | o    | o   |
| 3.....     | 0.05                 | +    | ..... | +    | +    | +   | o    | o   | o    | o   |
| 4.....     | 0.10                 | +    | +     | +    | +    | +   | sl.  | sl. | o    | o   |
| 5.....     | 0.30                 | +    | +     | +    | +    | +   | +    | +   | +    | sl. |
| 6.....     | 0.50                 | +    | +     | +    | +    | +   | +    | +   | o    | +   |
| 7.....     | 1.00                 | +    | +     | +    | +    | +   | +    | +   | +    | sl. |

TABLE X  
RELATIVE NUMBER OF BACTERIA IN CULTURE SOLUTIONS  
BASED ON CULTURE A1 = 100, EXPERIMENT 5

| IRON (MG.) | SODIUM CITRATE (GM.) | A    | B     | C    | D    | E     | F     | G     | H     | I     |
|------------|----------------------|------|-------|------|------|-------|-------|-------|-------|-------|
|            |                      | 0.01 | 0.02  | 0.03 | 0.05 | 0.1   | 0.15  | 0.2   | 0.25  | 0.3   |
| 1.....     | 0.01                 | 100  | 33    | 25.4 | 7.0  | 1.68  | ..... | ..... | ..... | ..... |
| 2.....     | 0.02                 | 30   | 13.6  | 18.0 | 3.6  | ..... | ..... | ..... | ..... | ..... |
| 3.....     | 0.05                 | 7.2  | ..... | 18.9 | 8.1  | 11.7  | 3.6   | 3.8   | ..... | ..... |
| 4.....     | 0.10                 | 32.4 | 21.6  | 13.5 | 7.2  | 4.5   | 4.3   | 6.3   | 3.6   | ..... |
| 5.....     | 0.30                 | 25.2 | 23.4  | 19.1 | 18.2 | 5.4   | 6.6   | 9.9   | 17.1  | 4.4   |
| 6.....     | 0.50                 | 14.0 | 9.0   | 14.4 | 16.6 | 7.2   | 3.96  | 4.5   | 3.3   | ..... |
| 7.....     | 1.00                 | 16.7 | 5.94  | 8.1  | 6.7  | 5.4   | 7.5   | 16.7  | 7.2   | 8.1   |

With some irregularities there is a depression in growth in each series as the amount of citrate per culture increases, and the amount of citrate at the growth extinction point is greater as the amount of iron is increased. The data for the growth extinction points are given in table XI, and in fig. 5 the relation of citrate to growth is shown for cultures containing 0.01 mg. total iron.

In one respect the results differ from those obtained in the experiments with *Chlorella*. At the low citrate contents growth is depressed as the iron content increases. The explanation of this is not

clear at the present time. While it should be emphasized that the experiment in this case is not exact because of the uncontrolled

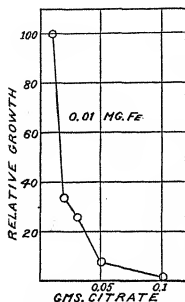


FIG. 5.—Effect of citrate on growth of bacteria in solutions having 0.01 mg. total iron (experiment 5).

purity of the cultures, it suggests that with certain bacteria the iron ion is an important factor in growth. The experiment should be repeated under controlled conditions with a pure culture of known species of bacteria.

It is of interest in this connection that WEBSTER (16) found that the growth of *Bacterium leptisepticum* was favored by addition to culture medium of iron in the complex form  $\text{Fe}(\text{OH})_2(\text{NC})_5\text{Na}_3$ . He obtained optimum growth at a concentration of 0.002 mg. per cc.

### Iron-ion concentration theory

#### I. STRUCTURE OF COMPLEX IRON-CITRATE COMPOUND

An explanation of the results thus far described was sought in the theory of complex-ion formation as set forth by STIEGLITZ (7). Due credit should be given to USPENSKI, whose work has already been dis-

TABLE XI

EXTINCTION POINT FOR GROWTH OF BACTERIA IN  
EXPERIMENT 5 AT VARIOUS IRON  
CONCENTRATIONS

| MG. FE PER CULTURE | GM. CITRATE PER CULTURE WHERE JUST NO GROWTH |
|--------------------|--|
| 0.01.....          | 0.05   |
| 0.02.....          | 0.075  |
| 0.05.....          | 0.125  |
| 0.10.....          | 0.20   |
| 0.30.....          | 0.30   |
| 0.50.....          | > 0.30                                       |
| 1.00.....          | > 0.30                                       |

cussed, for the application of the idea of complex-ion formation to culture solutions.

In the present work it was observed that, when sodium or potassium citrate was added to culture solutions containing iron, the color of the solutions deepened, and that if sufficient iron were present an intense yellowish green color resulted. This suggested the formation of a distinct chemical substance in the culture solution. Pure solutions of ferric chloride and sodium citrate when mixed gave rise to this same color. This color then was assumed to be that of the complex sodium ferric citrate in which the iron is present in the anion.

Careful tests were made, using equimolecular solutions of potassium citrate and ferric chloride. It was found at a concentration of 1.5 mols of citrate to 1.0 of  $\text{FeCl}_3$  that: (1) the brownish color due to the latter just disappeared and the solution turned yellowish green; and (2) the ordinary chemical reactions for iron ions just faded out. This was taken to be an indication of the composition of the complex potassium iron citrate. The presence of iron in the anion explains the fact that the usual reactions for iron ions are not given. Physico-chemical experiments were then made to establish this with more certainty.

EXPERIMENT 6: FREEZING POINT DETERMINATIONS ON IRON-CITRATE MIXTURES.—A series of freezing point determinations was carried out on various mixtures of ferric chloride and sodium citrate. Two solutions were prepared, 0.1 M  $\text{FeCl}_3$  and 0.1 M sodium citrate. They were then mixed so as to give varying ratios of iron to citrate. In this way the concentration of the solution was always 0.1M (total salt). The data are given in table XII and shown graphically in fig. 6. A sharp break in the graph occurs when the ratio of iron to citrate is 1:1.5. Delta is minimum at this point. It was observed directly after mixing these solutions that no. 1 had a somewhat deep brown color, no. 2 had a lighter brown color, while no brown color could be detected in no. 3 which was yellowish green. This minimum in the freezing point curve and the observations on color again indicate that the complex compound has a composition corresponding to the ratio 1:1.5, or two molecules of ferric chloride to three of sodium citrate, since one would conclude that the total number of ions is minimum at this composition.

EXPERIMENT 7: CONDUCTIVITY DETERMINATIONS ON IRON-CITRATE MIXTURES.—Solutions were prepared in the same manner

as used in experiment 6 and electrical conductivity determinations were made. In this case, however, the total molecular concentration

TABLE XII  
FREEZING POINT DEPRESSIONS OF MIXTURES OF FERRIC CHLORIDE  
AND SODIUM CITRATE, EXPERIMENT 6

| SOLUTION NO. | Cc. 0.1 M<br>FeCl <sub>3</sub> | Cc. 0.1<br>NA CITRATE | RATIO<br>Fe:CITRATE | Δ<br>SOLUTION |
|--------------|--------------------------------|-----------------------|---------------------|---------------|
| 1.....       | 14                             | 7                     | 1:0.5               | 0.719         |
| 2.....       | 10                             | 10                    | 1:1                 | 0.672         |
| 3.....       | 10                             | 15                    | 1:1.5               | 0.642         |
| 4.....       | 7                              | 14                    | 1:2                 | 0.644         |
| 5.....       | 6                              | 15                    | 1:2.5               | 0.654         |
| 6.....       | 5                              | 15                    | 1:3                 | 0.648         |
| 7.....       | 5                              | 20                    | 1:4                 | 0.651         |
| 8.....       | 4                              | 20                    | 1:5                 | 0.654         |
| 9.....       | 3                              | 18                    | 1:6                 | 0.651         |
| 10.....      | 20                             | 0                     | .....               | 0.670         |
| 11.....      | 0                              | 20                    | .....               | 0.656         |

was adjusted to M/100. An Ostwald conductivity cell with a vacuum jacket was used and the temperature was adjusted to ap-

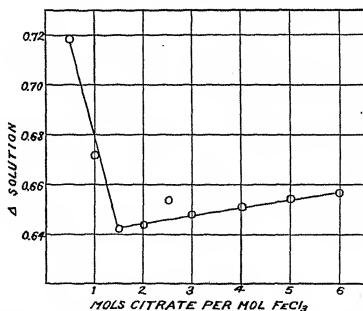


FIG. 6.—Freezing point determinations of mixtures of ferric chloride and sodium citrate (experiment 6).

proximately 25.5° C. for each measurement. The actual conductance of the cell was calculated in each case from the readings to reciprocal ohms (mhos). Although the cell factor was determined to be 0.395



it was not deemed necessary for the purposes of this experiment to calculate the specific conductivity. The results are given in table

TABLE XIII  
ELECTRICAL CONDUCTIVITIES OF MIXTURES OF FERRIC  
CHLORIDE AND SODIUM CITRATE, EXPERIMENT 7;  
TOTAL MOLAR CONCENTRATION M/100

| SOLUTION NO. | RATIO<br>FE:CITRATE | t° C. | $C = 1/V$<br>$\times 10^4$ MHOS |
|--------------|---------------------|-------|---------------------------------|
| 1.....       | 1:0.5               | 25.5  | 96.07                           |
| 2.....       | 1:1                 | 25.5  | 71.83                           |
| 3.....       | 1:1.5               | 25.5  | 49.09                           |
| 4.....       | 1:2                 | 25.4  | 48.37                           |
| 5.....       | 1:2.5               | 25.5  | 50.83                           |
| 6.....       | 1:3                 | 25.6  | 53.38                           |
| 7.....       | 1:4                 | 25.6  | 55.77                           |
| 8.....       | 1:5                 | 25.6  | 56.74                           |
| 9.....       | 1:6                 | 25.5  | 57.73                           |
| 10.....      | 1:0                 | 25.4  | 108.30                          |
| 11.....      | 0:1                 | 25.4  | 62.87                           |

XIII and fig. 7. As in the case of the freezing point determinations, a sharp break occurs in the electrical conductivity data when the ratio

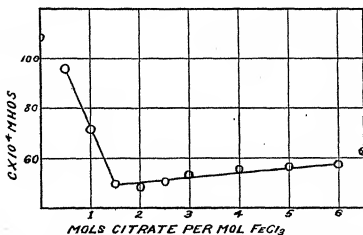


FIG. 7.—Electrical conductivity determinations on mixtures of ferric chloride and sodium citrate; points not connected represent values for single components  $\text{FeCl}_3$  and  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$  (experiment 7).

of iron to citrate is 1:1.5. The conductivities were carefully checked the next day on the same solutions with the same result.

EXPERIMENT 8: CONDUCTIVITY TESTS ON IRON-CITRATE MIXTURES.  
—In this case experiment 7 was repeated, using potassium citrate

instead of the sodium salt. The same disappearance of the brown color due to the ferric chloride was also noted at the ratio of 1:1.5 in

TABLE XIV  
ELECTRICAL CONDUCTIVITIES OF MIXTURES OF FERRIC  
CHLORIDE AND POTASSIUM CITRATE, EXPERIMENT 8;  
TOTAL MOLAR CONCENTRATION M/100

| SOLUTION NO. | RATIO<br>FE:CITRATE | t°C. | C=i/v<br>X10 <sup>4</sup> MHOS |
|--------------|---------------------|------|--------------------------------|
| 1.....       | 1:0                 | 25.0 | 110.10                         |
| 2.....       | 1:0.5               | 25.0 | 104.10                         |
| 3.....       | 1:1.0               | 25.0 | 76.05                          |
| 4.....       | 1:1.5               | 25.0 | 57.74                          |
| 5.....       | 1:2.0               | 25.0 | 59.76                          |
| 6.....       | 1:2.5               | 25.0 | 63.13                          |
| 7.....       | 1:3.0               | 25.0 | 65.03                          |
| 8.....       | 1:4.0               | 25.0 | 69.20                          |
| 9.....       | 0:1                 | 25.0 | 81.83                          |

this experiment. The results obtained are given in table XIV and fig. 8. The same conductivity cell was used. Again a sharp break occurs at the ratio 1:1.5.

EXPERIMENT 9: CONDUCTIVITY TESTS ON IRON-CITRATE MIXTURES.—This experiment was a repetition of experiment 8. The results are given in table

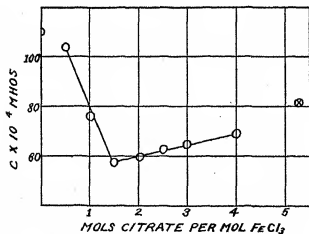


FIG. 8.—Electrical conductivity determinations on mixtures of ferric chloride and potassium citrate; points not connected represent values for single components FeCl<sub>3</sub> and K<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> (experiment 8).

experiment 8. The results are given in table XV and fig. 9, where the bridge readings only are given, with a constant resistance in the resistance box of 100 ohms. In this case two extra solutions were introduced in the vicinity of the 1:1.5 ratio. The results check with the previous ones, showing the complex compound to be formed at this ratio of iron to citrate.

EXPERIMENT 10: FIRST PREPARATION OF COMPLEX COMPOUND.—It was thought that by preparing the complex compound in a pure

state and then analyzing the preparation obtained chemically, the composition could be established with still greater certainty. A preliminary test showed that when acetone is added to a mixture of potassium citrate and ferric chloride a two-layer system is formed, with a green solution below and a practically colorless layer above. Further, if equal volumes of the solution and acetone were used the lower layer was only about one-fourth of the total volume. It was assumed: (1) that the acetone extracts water from the solution; (2) that the green complex compound is much more soluble in the lower (mainly aqueous phase); and (3) that the KCl formed in the reaction would be soluble in the upper layer of water and acetone. By repeated extraction, therefore, the complex compound could be purified. The following solutions were prepared: (1) M/1  $\text{FeCl}_3$ ; (2) M/1  $\text{K}_3\text{C}_6\text{H}_5\text{O}_7$ .

To 50 cc. of (1) 75 cc. of (2) was added slowly. At first the solution turned a deep brown, then a greenish brown, and finally, as the last few drops of the citrate were added it was observed that the last trace of brown disappeared. A clear deep green solution resulted. The solution was then placed in a separatory funnel with about one and one-half volumes of acetone, shaken, the two layers allowed to separate, and the lower one containing the complex run into a flask. It was diluted to 100 cc. with water and again extracted with acetone as before. This process was repeated five times. At this time the solution no longer gave a test for chlorides, showing that all the KCl had been removed. After each extraction a drop of the solution was placed on a glass slide and allowed to dry. At first crystals of KCl separated, but after several extractions the drop dried to a clear

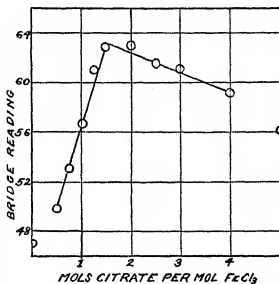


FIG. 9.—Electrical conductivity determinations on mixtures of ferric chloride and potassium citrate; points not connected represent values for single components  $\text{FeCl}_3$  and  $\text{K}_3\text{C}_6\text{H}_5\text{O}_7$ .

brittle mass which began to crack spontaneously in a dendriform manner. As the water was removed by the acetone the solution with the compound in it became very viscous. The substance when dried has the appearance of resin and shows radiating cracks as already mentioned. It is completely soluble in water, forming a yellowish green solution which when dilute is more of a yellow color.

Two 10 cc. aliquots of a solution containing 1.0767 gm. per 100 cc. were evaporated to dryness and ashed. One was analyzed for Fe and the other for K. The results were 15.92 per cent Fe and 16.50 per cent K.

TABLE XV

ELECTRICAL CONDUCTIVITIES OF MIXTURES OF FERRIC CHLORIDE AND POTASSIUM CITRATE, EXPERIMENT 9; TOTAL MOLAR CONCENTRATION M/100 (CONSTANT RESISTANCE OF 100 OHMS USED IN RESISTANCE BOX)

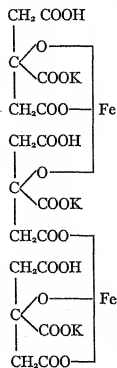
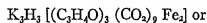
| SOLUTION NO. | RATIO<br>Fe:CITRATE | SOLUTION COLOR      | AVERAGE BRIDGE<br>READING AT<br>R=100 OHMS<br>t=25° C. |
|--------------|---------------------|---------------------|--|
| 1.....       | 1:0                 | Brown               | 4701   |
| 2.....       | 1:0.5               | Lighter brown       | 4981   |
| 3.....       | 1:0.75              | Still lighter brown | 5297   |
| 4.....       | 1:1.0               | Still lighter brown | 5668   |
| 5.....       | 1:1.25              | Still lighter brown | 6098   |
| 6.....       | 1:1.5               | Greenish yellow     | 6283   |
| 7.....       | 1:2.0               | Greenish yellow     | 6300   |
| 8.....       | 1:2.5               | Greenish yellow     | 6154   |
| 9.....       | 1:3.0               | Greenish yellow     | 6111   |
| 10.....      | 1:4.0               | Greenish yellow     | 5918   |
| 11.....      | 0:1                 | Colorless           | 5612   |

EXPERIMENT 11: SECOND PREPARATION OF COMPLEX COMPOUND.— In this experiment another method was used for the preparation of the complex potassium-iron citrate. The following mixture was prepared: 26 cc. M/1  $\text{FeCl}_3$  + 37.5 cc. M/1  $\text{K}_2\text{C}_6\text{H}_5\text{O}_7$ . In these proportions an excess of ferric chloride should be present according to the 1:1.5 ratio previously determined, and in fact a strong ferric sulphocyanate test was given by the solution. The mixture was then evaporated to dryness and extracted with a mixture of equal parts of absolute alcohol and ether until no test for ferric iron was found. It was then necessary to remove the KCl which had been formed. This was effected by extracting in a Soxhlet apparatus with 95 per cent alcohol, in which the KCl is slightly soluble and in which

the complex is practically insoluble. About one week of continuous extraction was required to remove the last trace of chlorides. After the alcohol was removed by evaporation at  $110^{\circ}$  C. a yellowish green powder remained which weighed approximately 7.3 gm.

The following tests were made on this powder. One gm. was found to be perfectly soluble in about 30 cc. of water at room temperature. There was no residue and a deep yellowish green solution resulted. A 1 per cent solution was found to be strongly acid to litmus, acid to methyl red, and to have a pH value of about 4.2 with brom phenol blue. An extremely slight test for ferric iron was given, using the sulphocyanate test even when the ferric sulphocyanate was dissolved in an amylc layer according to the method of STOKES and CAIN (8). Chlorides were absent. A test with potassium ferricyanide gave a deep blue color but no precipitate, showing ferrous iron to be present but in small amount. An analysis of the preparation gave 14.83 per cent Fe and 14.06 per cent K.

Many structural formulas were constructed, based on the data presented in this paper and on the theory of complex ion formation. The only one which gave agreement with these results is the following:

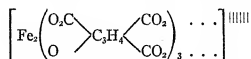


The agreement between this constitution and the analytical data is shown as follows:

|                    | CALCULATED | OBTAINED         |                  |
|--------------------|------------|------------------|------------------|
|                    |            | Experiment<br>10 | Experiment<br>11 |
| Percentage Fe..... | 14.03      | 15.92            | 14.83            |
| Percentage K.....  | 14.73      | 16.50            | 14.06            |

The results check rather well with that calculated from the preceding formula, especially for experiment 11 in which the method of preparation also appeared to be more satisfactory. That there are free carboxyl groups as shown is indicated by the acid reaction.

It was found after performing these experiments and working out the structure given, that BELLONI (4) has determined the structure of the sodium salt to be analogous. He also found that the complex is formed when the ratio of iron to citrate is 1:1.5, and considers that all but the sodium and acid hydrogen atoms constitute a hexavalent complex ion of which the complex compound is the trisodium salt. The structure of this complex ion is given as:



BELLONI further furnished evidence that the iron is attached to three of the carboxyl groups, by showing that salts of this complex containing more than three  $\text{NH}_4$  groups are not obtained.

Attempts to determine the molecular weight of the compound have so far proved unsuccessful because, while the substance is very soluble in water in which it is dissociated to an unknown extent, it is practically insoluble in all of the other solvents tried. For instance, it was found to be insoluble in chloroform, aniline, toluene, methanol, ethanol, benzine, amyl alcohol, carbon tetrachloride, alcohol ether, and glacial acetic acid. While it appeared to be very soluble in glycerin this was not considered to be a suitable solvent for molecular weight determinations. This insolubility in various reagents also checked attempts to further purify the compound by crystallization.

Negative results were obtained in practically all cases in trials to precipitate the complex ion as the salt of a heavy metal. The following ions gave no precipitate nor change in color in an aqueous solu-

tion of the complex: Ag, Ba, Ca, Cu, Al, Hg, Mn, Cd, Cr, Ni, Zn, Mg, Sr, and Sn. Cobalt gave no precipitate but the solution turned brown. Lead, both as acetate and as nitrate, gave an abundant precipitate but when this was filtered off the filtrate, which at first was a clear green color, after standing formed more precipitate. Therefore purification in this manner did not appear to be possible.

## II. FERRIC AND FERROUS IONS

It has been shown in connection with the experiments on *Chlorella* (experiment 1a), that when the citrate concentration is increased in a solution containing iron there occurs a decrease in the sulphocyanate reaction for ferric iron. It was also shown in experiment 11 that the complex compound shows a very slight reaction for ferric iron, and a rather definite test for ferrous iron. The fact that ferrous iron is present in such solutions is no doubt of great importance and the subject will be discussed later. It can be assumed that it is formed through the reducing action of the citrate.

For the present we may consider the effect of complex-ion formation on these reactions. If the complex formed is a somewhat stable one, we should expect these reactions to fade out at the point of complex formation, or at least to be very faint. That such is the case will be seen from the following experiments.

EXPERIMENT 12: FERRIC-ION REACTION IN IRON-CITRATE MIXTURES.—In this case only the ferric-ion reaction was studied. Molar solutions of ferric chloride and potassium citrate were mixed in such proportions as to give the ratios shown in table XVI, where the colors of the solutions and the sulphocyanate reactions after dilution are also given. The reaction for ferric iron is seen to fade out at the ratio 1:1.5.

EXPERIMENT 13: FERROUS AND FERRIC-ION REACTIONS IN IRON-CITRATE MIXTURES.—This time the reactions for both ferric and ferrous ions were followed, the latter by means of potassium ferri-cyanide. The same procedure as used in experiment 12 was employed. The results are given in table XVII. As before, the reaction for ferric ions fades out at the ratio 1:1.5. The reaction for ferrous iron is interesting, as a very strong test is given at the ratio 1:0.5, where that for ferric ions is also strong, and then as the amount of

citrate is increased the reaction decreases. It finally fades out, as does the reaction for ferric iron, but at a somewhat greater citrate concentration. What is taking place as the citrate ion increases may be considered to be: (1) ferric iron is partly reduced to ferrous iron;

TABLE XVI

EFFECT OF CITRATE ON REACTION FOR FERRIC IRON,  
EXPERIMENT 12

| SOLUTION NO. | RATIO<br>FE:CITRATE | COLOR      | Fe(SCN) <sub>3</sub> REACTION<br>ON DILUTED SAMPLE |
|--------------|---------------------|------------|--|
| 1.....       | 1:1.0               | Brown      | Deep blood red                                     |
| 2.....       | 1:1.4               | Pale brown | Cherry red   |
| 3.....       | 1:1.5               | Green      | Faint red  |
| 4.....       | 1:1.6               | Green      | Green  |
| 5.....       | 1:1.8               | Green      | Green  |
| 6.....       | 1:2.0               | Green      | Green  |

(2) both are converted into the complex; and (3) both ferrous and ferric ions are further depressed by an excess of citrate. The ratio  $[Fe...]/[Fe...]$  may therefore be expected to change with increasing citrate from a low value to a high one, but in a manner difficult to predict except from quantitative data. In solutions in which the cit-

TABLE XVII

EFFECT OF CITRATE ON REACTIONS FOR FERRIC IRON  
AND FERROUS IRON, EXPERIMENT 13

| SOLUTION NO. | RATIO<br>FE:CITRATE | TESTS KSCN | WITH<br>K <sub>3</sub> Fe (CN) <sub>6</sub> |
|--------------|---------------------|------------|---|
| 1.....       | 1:0.5               | Strong     | Very strong                                 |
| 2.....       | 1:1.0               | Good       | Strong                                      |
| 3.....       | 1:1.5               | .....      | Good  |
| 4.....       | 1:2.0               | .....      | .....                                       |
| 5.....       | 1:3.0               | .....      | .....                                       |

rate is greater than that called for by the 1:1.5 ratio the total iron-ion concentration, ferrous and ferric, will be very small; and the ratio of ferrous ions to ferric ions, although perhaps large, may not have a finite value.

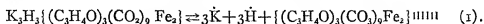
### III. EQUILIBRIUM CONDITIONS OF POTASSIUM IRON-CITRATE COMPLEX

An understanding of the relation of the theory of complex-ion formation to the various facts presented in this work will be en-



hanced by a consideration of the equilibrium conditions which should be expected in such solutions. For the purposes of discussion it is assumed that only ferric ions are formed, although as shown ferrous ions are also present.

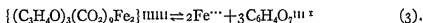
By ordinary ionization the complex iron citrate compound  $K_3H_3\{(C_3H_4O)_3(CO_3)_3Fe_2\}$  breaks up as follows:



The mass law equation for this is:

$$\frac{[K]^3 \cdot [H]^3 \cdot [\{(C_3H_4O)_3(CO_3)_3Fe_2\}^{|||||}]}{[K_3H_3\{(C_3H_4O)_3(CO_3)_3Fe_2\}]} = K_{\text{ionization}} \quad (2),$$

where  $K_{\text{ionization}}$  is the ionization constant of the complex compound itself. The complex anion formed in this dissociation, however, also ionizes to a slight but appreciable extent into iron ions and citrate ions, so that we have:



The mass law equilibrium in this case is:

$$\frac{[Fe \cdots]^2 \cdot [C_6H_4O_7^{|||}]^3}{[\{(C_3H_4O)_3(CO_3)_3Fe_2\}]} = K_{\text{instability}} \quad (4),$$

where  $K_{\text{instability}}$  is the dissociation constant of the complex ion. This constant has a very small value, showing stability of the complex anion to be high. The numerical value is not known but its relative magnitude can be deduced from data already given.

It is with equation (4) that we are principally concerned. The effect of various factors on this equilibrium will therefore be considered.

The solubility of iron at alkaline reactions when citrate is present, as is described in a previous paper (HOPKINS and WANN 6), may be explained by these equations. The stability of the complex is such

<sup>1</sup> The reaction is probably not so simple as shown in equation (3), and other reactions may occur which give rise to true citrate ions  $C_6H_5O_7^{|||}$  instead of  $C_6H_4O_7^{|||}$  as shown. For the purposes of the present discussion the reactions as shown are considered to be sufficient.

that the concentration of iron ions is very small; hence insoluble compounds like  $\text{Fe}(\text{OH})_3$  or  $\text{FePO}_4$  will not form, since their solubility products are greater than this value. The higher the citrate content, therefore, the less is the tendency for these compounds to form and remove the iron from solution.

The effect of changing citrate concentration on growth of *Chlorella* can now be explained as well. Consider a culture solution in which iron and citrate are in such proportions as just to form the complex. Most of the iron will then be present in the complex anion, but at the same time there will be present a small but sufficient concentration of iron ions for a certain amount of growth to be possible. This is due to the slight ionization of the complex anion as shown in equation (3). As the concentration of the citrate ion is increased, the concentration of the iron ions must decrease in order to maintain  $K$  instability constant. The growth of *Chlorella* will then be depressed, let us say, due to this decrease in iron ions. If the citrate is further increased the iron-ion concentration, although small, will be depressed and continue to decrease until finally a point will be reached where there are insufficient iron ions present for growth to be possible.

The reduction of the toxicity of large amounts of iron in culture solutions containing citrate, as reported by USPENSKI (11) for *Volvox*, and as indicated by the tolerance of *Chlorella* to large amounts of total iron as reported previously (6), can also be explained on the basis that the active iron or iron-ion concentration has been reduced to such an extent that there is no injury.

The effect of changing the hydrogen-ion concentration may be partly explained on this basis. If the hydrogen-ion concentration is increased it is evident, since citric is a weak acid, that the citrate ion will be depressed, and hence the iron-ion concentration must increase in order to maintain the constancy of  $K$  instability in equation (4). This would then cause certain changes in growth apart from the effect of the  $\text{H}^+$ -ion per se, and it may even be that the inhibition of growth at low pH values is partly due to this increase in the iron-ion concentration which is toxic. The converse effect would be brought about at high pH values, and it is therefore suggested that the final depression and inhibition of the growth of *Chlorella* as the

OH-ion concentration is increased. (WANN and HOPKINS 12) may be due partially at least to its effect in reducing the iron-ion concentration to a point where growth will not occur. Thus the effect of pH may not be direct but indirect.

### Discussions and conclusions

The results thus far obtained, as recorded in this paper and in the previous work already mentioned, appear to make the whole matter of the relation of iron to culture solutions and to growth much clearer by explaining many diverse results on one fundamental basis, namely, that of the theory of active iron or iron-ion concentration. It is hoped, however, that this idea may have even wider application than to the iron problem alone, important as this is. The suggestion is ventured that other metals which form such complexes with organic acids will be found to behave in an analogous manner. The elements calcium and manganese might be mentioned. The rôle of the former in the clotting of blood, for instance, is well known. It is also true that citrates will prevent blood clotting, and that calcium forms a complex with citrates. A logical explanation in this case may therefore be that citrates bring about this effect by depressing the calcium-ion concentration. In the case of blood leucocytes, Miss WOLF (15) found that calcium was the only inorganic ion to be positively chemotactic, but when in the form of calcium citrate she states that the negative chemotaxis of the citrate ion neutralizes the positive effect of the calcium. A better explanation would perhaps be that the calcium ion is depressed through the formation of a complex calcium-citrate anion.

In regard to iron, its solubility at high pH values in the presence of citrates is made clear, and undoubtedly this holds for other organic acids and compounds. This fact may also be applied to the availability of iron in soils at high pH values when organic matter is present, and to its unavailability when it is absent.

The relative availability claimed for iron furnished in different forms may be nothing more than a comparison of the varying solubilities of the iron in these salts in the culture solutions used, or, assuming the iron to be in solution, a comparison of the varying values for the iron-ion concentration occurring under these conditions.

However, the effect of a change in the ratio ferrous to ferric iron should not be lost sight of. The case is analogous to various physiological effects attributed, in the older literature, to different acids which we now know may be explained largely on the basis of the hydrogen-ion concentration involved.

This idea will undoubtedly have an important bearing on conditions within the living cell where, due to the organic substances present, we should expect complex-ion formation to take place. Changes in the metabolism of the cell may thus be expected to change the iron-ion concentration and modify physiological responses, just as changes in the external solution by which the cells are bathed might do. What these intracellular complexes containing iron are is not well understood. ANSON and MIRSKY (1, 2) have called attention to the wide distribution in living cells of certain iron-pyrrol complexes known as heme compounds, to which they assign a rôle in biological oxidations on the basis of the theory of WARBURG (13, 14). These complexes may have a function in regulating the intracellular iron-ion concentration, which in turn is undoubtedly affected by that of the external solution.

In regard to cellular respiration, if iron has the importance assigned to it by WARBURG it would be natural to assume, on the basis of the ideas presented in this paper and on general physico-chemical considerations, that it is the active or iron-ion concentration and not the total iron which is the essential catalyst in cellular oxidation. Since the iron-ion concentration has been shown here profoundly to affect growth, which is one of the manifestations of respiration, it seems logical to attach the importance to it rather than to the total iron.

### Summary

1. Studies on the growth of *Chlorella* sp. in culture solutions containing iron salts with sodium or potassium citrate in many different combinations indicate that iron is effective physiologically in the form of its ions. The total soluble iron may vary within wide limits and still have the same effect, either on growth or toxicity, providing the iron-ion concentration is the same. Certain observations point to the fact that some species of bacteria behave similarly.

2. The effect of citrates on growth in solutions containing a constant amount of total iron is paralleled by their effect on the relative iron-ion concentration as determined by chemical tests.

3. A theory relating iron-ion concentration to growth based on complex-ion formation has been developed which makes clear many problems involved in culture solution work, in the field and in biological phenomena in general.

4. The iron-ion concentration necessary for the growth of *Chlorella* is small. A provisional value at which growth may just take place has been calculated to be  $1.47 \times 10^{-6}$  gram ions.

5. It is suggested that the iron-ion concentration plays an important rôle in many physiological processes, especially those involving cell metabolism such as biological oxidations, and that these processes should be reconsidered in their relation to the iron-ion concentration rather than to the total iron.

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#### LITERATURE CITED

1. ANSON, M. L., and MIRSKY, A. E., The heme compounds in nature and in biological oxidations. *Science N.S.* 68:647-648.
2. ———, Heme and tissue iron. *Jour. Gen. Physiol.* 12:401-405. 1929.
3. BAUDISCH, O., and WELO, L. A., On the aging of natural waters. *Jour. Biol. Chem.* 64:771-779. 1925.
4. BELLONI, E., I sali organico del ferro. II. Citrati ferrici e citrati ferrici ammoniacali. *Gazz. Chim. Ital.* 50:II:159-212. 1920.
5. HOPKINS, E. F., and WANN, F. B., Relation of hydrogen-ion concentration to growth of *Chlorella* and to the availability of iron. *BOT. GAZ.* 81:353-376. 1926.
6. ———, Iron requirement for *Chlorella*. *BOT. GAZ.* 84:407-427. 1927.
7. STIEGLITZ, JULIUS, The elements of qualitative chemical analysis. Vol. I. pp. 312. New York. 1911.
8. STOKES, H. N., and CAIN, J. R., On the colorimetric determination of iron with special reference to chemical reagents. *Bur. Standards Bull.* 3. 115-156. 1907.
9. USPENSKI, E. E., Contributions to the study of the action of different quantities of iron. Solutions acting as a buffer to iron. *M.S.S.R. Trans. Inst. Fertilizers No. 23.* Moscow 1924 (Russian with English résumé).

10. USPENSKI, E. E., Eisen als Faktor für die Verbreitung niederer Wasserpflanzen. Pflanzenforschung herausgegeben von R. Kolkwitz h. 9, pp. 104. Jena. G. Fischer. 1927.
11. USPENSKI, E. E., and USPENSKAJA, W. J., Reinkultur und Ungeschlechtliche Fortpflanzung des *Volvox minor* und *Volvox globator* in einer synthetischen Nährlösung. Zeit. Bot. 17:273-308. 1925.
12. WANN, F. B., and HOPKINS, E. F., Further studies on growth of *Chlorella* as affected by hydrogen-ion concentration. BOT. GAZ. 83:194-201. 1927.
13. WARBURG, OTTO, Iron, the oxygen carrier of respiration ferment. Science N.S. 61:575-582. 1925.
14. ———, The chemical constitution of respiration ferment. Science N.S. 68: 437-443. 1928.
15. WOLF, E. P., Experimental studies on inflammation. I. The influence of chemicals upon the chemotaxis of leucocytes in vitro. Jour. Exp. Med. 34: 375-396. 1921.
16. WEBSTER, L. T., Changes in the virulence and growth characteristics of *Bacterium leptisepticum* following alterations in oxygen tension. Soc. Exp. Biol. & Med. Proc. 22:139-141. 1924-5.

# HOURLY VARIATIONS IN CARBOHYDRATE CONTENT OF LEAVES AND PETIOLES<sup>1</sup>

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 401

HARRY F. CLEMENTS

(WITH EIGHTEEN FIGURES)

## Introduction

There are few fields in biological work which offer greater obstacles to investigation than that phase of plant physiology which deals with the photosynthetic activities of plants. This is especially emphasized when it is remembered that nearly a century ago the granule of starch in the chloroplast was observed as distinct from the chloroplast itself, and only a few years later it was determined that starch was formed by green plants in the sunlight. Still later it was discovered that some simpler form of carbohydrate is the forerunner of starch. And now, nearly a century later, little more has been learned about the process itself. Extremely interesting and in many cases important data have been obtained by the hundred and more workers who have reported since that time regarding the fluctuations of the carbohydrates in the leaves of a great variety of plants. The quantitative results of some of the earlier workers have been challenged on the grounds of unreliable procedures, but although more evidence has been accumulated through the use of modern and accurate methods, the conclusions drawn by the earlier workers remain.

The German workers have been most active in this field. A large share of their work has been done on a purely microchemical basis, with special emphasis on attempts to follow the details of starch formation. This has resulted in extremely valuable information, but the fact remains that where starch alone is observed only a portion of the synthetic process is studied. Starch will not accumu-

<sup>1</sup> The investigation reported in this paper was carried on by the writer as Assistant in Plant Physiology in the Botanical Section of the Michigan Agricultural Experiment Station at East Lansing, Michigan. By permission of Director V. R. GARDNER, the paper is published in the *BOTANICAL GAZETTE*.

late in all plants, and even where it is found to occur abundantly it does not do so if the soluble carbohydrates are able to move from the leaves as rapidly as they are formed. Thus, when starch deposition alone is observed only an incomplete study of the entire carbohydrate synthesizing system can be made, since much of the sugar formed disappears from the leaves before it is changed to starch. In other words, such observations interpret only the accumulation of carbohydrate materials in the leaf.

It is desirable to know, however, as the recent work of LÄNGNER (18), ALEXANDROV (1), and others shows, that starch is deposited systematically in the various tissues of the leaves. Thus, LÄNGNER has demonstrated that the successive layers of the spongy tissue become filled with starch after the latter has accumulated in the palisade layer. This continues inward, and at the end of the day the starch disappears first from the palisade layer followed by those of the spongy tissues most distant from the vascular bundle. ALEXANDROV has shown that there usually is some starch present in the sheath around the bundle.

The three principal contributions by the English have been purely quantitative. The quantitative method, although it yields results which portray the types of carbohydrates and their relative amounts in plant tissue, does not yield information regarding the distribution of these compounds within specific tissues or cells. Thus in the leaf it is possible to have certain forms concentrated in the cells of the bundle sheath, others in the palisade tissues, and still others in the minor veins; and yet its analysis as a whole would show only relative totals.

Inadequate though each method of attacking this important problem of carbon assimilation may be, each has contributed information furnishing clues and suggestive interpretations. Until a convenient combined use of microchemistry and macrochemistry is developed, much can be learned of the synthetic processes of plants by the use of these methods separately.

#### Historical data

VON MOHL (22) was the first to notice that the chloroplast and the granule of starch were really two entities, and that starch was formed



by the chloroplast. Apparently this observation escaped the immediate attention of scientists, for it was not until 1862 that SACHS (29) made the far-reaching generalization that starch was formed in the chloroplasts, and also that it was formed there in the presence of light and was removed at night. In 1870 the BAEYER (2) theory of carbon assimilation was announced. Later SACHS (30) stated that materials move out of the leaves both day and night, and that normally leaves are relieved of the photosynthate during the day and only the accumulated excess is removed at night. He called attention to the fact that conditions are more favorable for translocation in the warmer hours of the day than they are at night when it is cooler.

MEYER (20) pointed out that starch formation was not universal in plants, which observation had been previously recorded by BOEHM (5), who worked with a number of plants. SCHIMPER (31) repeated the earlier experiments on starch, and in addition showed that by floating leaves on a glucose solution starch may be formed in them even in the dark.

In the early works reviewed so far microchemical methods were used exclusively, but in 1893 BROWN and MORRIS (4) reported work in which quantitative methods were employed. Their ideas are briefly as follows: Because cane sugar is more abundant in the leaves and fluctuates in amount more than any other sugar, it is the first sugar formed; fructose is always in excess of glucose; maltose is present; cane sugar is hydrolyzed and translocated as dextrose and levulose while starch is moved as maltose and is formed directly from cane sugar.

During this period there was considerable speculation as to the form in which carbohydrates were translocated. BROWN and MORRIS claimed that glucose, fructose, and maltose were the forms. ROBERTSON, IRVINE, and DOBSON (27) were unable to find invertase in the roots of the sugarbeet, and concluded that sucrose must be moved as such from the stems and leaves. STRAKOSCH (35), using microchemical methods, found that dextrose was the only sugar which occurred in the palisade cells of the sugarbeet leaf.

CAMPBELL (6) suggested:

The reducing sugars are the first carbohydrates to be formed as soon as daylight begins. A little later the sucrose curve begins to rise, and later still the starch

curve. It would also appear that the cane sugar does not rise until the reducing sugars have reached their maximum which they maintain throughout the period of illumination. Similarly the starch does not rise in its turn until the cane sugar has reached its maximum.

STEPHANI (34) held that in view of the fact that reducing sugars are very scarce in the root of the beet, sucrose must be formed in the leaves. RUHLAND (28), however, thought that the sugars moved as simple sugars and were resynthesized. DELEANO (14) failed to crystallize any sucrose from the leaves of *Vitis vinifera*, and hence thought it absent. PELLET (25) reported that in the sugar cane glucose, fructose, and sucrose move together through the stem. PARKIN (24), working with *Galanthus nivalis*, indicated that because sucrose was more abundant and fluctuated in the leaves more than simple sugars it was the primary one; although as the leaves grow older and the season advances, sucrose as well as starch is less abundant, and the reducing sugars increase in amount.

DAVIS, DAISH, and SAWYER (12) reported on the sugar mangold, and DAVIS and SAWYER (13) on the potato. Some of their conclusions in respect to the mangold are: (1) Starch is present in the mangold leaves only during the very early stages of growth before extensive root development starts. (2) During the early life of the plant, when the leaves are developing rapidly, sucrose is present in the leaves in excess of the simple sugars. Later in the season, when storage is proceeding, the reverse is true. (3) Hexoses always predominate in the midribs and stalks. (4) Maltose is never present. (5) Pentoses are not abundant and are probably formed from hexoses and appear to be the precursors of the pentosans.

The conclusions of DAVIS and SAWYER in reference to the potato are: (1) When the tubers are beginning to develop, the principal sugar in the potato leaf is saccharose. Its amounts increase from sunrise up to 2:00 P.M. (2) The hexoses are present in amounts greater than 1 per cent of the dry weight. (3) Starch is apparently formed from hexoses. (4) In the stalks reducing sugars predominate. (5) Maltose is entirely absent from the potato.

MILLER (21), studying the relation between sorghum and corn, showed that although sucrose may be more abundant than the reducing sugars, this in itself is no argument favoring sucrose as the

first sugar of synthesis. TOTTINGHAM et al. (36) reported upon sugar variations in the leaves of the sugarbeet as affected by climatic variations.

PRIESTLEY (26) questioned the function generally assigned to sucrose. Using the data of DAVIS, DAISH, and SAWYER, he demonstrated that in reality the reducing sugars might be regarded as first sugars formed.

### Materials and methods

It is impossible to over-emphasize the care which must be exercised in all analytical work pertaining to carbohydrates. Every attempt was made and every precaution taken to insure comparable and reliable results.

Three species of plants were used, *Helianthus annuus*, *Soja max* var. Ito San, and *Solanum tuberosum* var. Russet Rural. These plants were selected for various reasons: (1) All of them are commonly grown as annuals in Michigan. (2) All three plants have a rather shallow and limited root system, probably rendering them sensitive to variations in moisture conditions. (3) As these plants were used for other nutritional work which will be recorded later, it was desirable to use one plant which stores its excess food mainly in the form of carbohydrate (potato); another which accumulates large reserves of proteins (soy bean); and a third which forms considerable amounts of oil (sunflower). (4) All three plants lend themselves well to analysis. (5) The plants are rather homozygous (this is true of the soy bean). The potatoes, for which the writer is indebted to Dr. E. J. KOTILA of Michigan State College, were multiplied from a single tuber selection. The sunflower is genetically a mixture, of course, but data presented later will show that the results are not greatly variable even for such a plant.

All three crops were planted in rows May 20, 1926, and May 22, 1927, in a sandy loam soil of some uniformity. The plants were located in Field 19 of the experimental fields of the Michigan State College Experiment Station near East Lansing, Michigan. Approximately three one-quarter-acre plots were used. Because of a dry period at seeding time it was necessary to replant the soy beans on May 30, 1926. From this time on the plants grew well. When nec-

essary, the plants were thinned to conform with commercial practice. The crops were cultivated three times.

Samples were collected at three different times each year. When a series was to be gathered, only a portion of each plot was used for sampling. For example, one row of sunflowers was selected for the work, in this way limiting the area from which the plants were selected and thus reducing soil variations to a minimum. When collections were made, 50 gm. of leaves and 50 gm. of petioles were gathered from each of the three species. It was the practice to gather the leaves which were but recently mature, which showed a decided thriftiness, and which were exposed to direct sunlight during the day. Usually only one leaf was picked from a single plant. Thus, in the case of the sunflower, a sample represented a composite of 8-10 plants; in the case of the soy bean, 45-50 plants; and in the case of the potato, 50-60 plants. In this way any fluctuation which occurs probably represents the average condition prevailing and not any individual difference.

In any particular series the material from all three plants was gathered once each hour for 24 consecutive hours. The routine was so arranged that not longer than 10 minutes elapsed after any one selection was made before the petioles and midribs had been severed from the leaves and both portions placed in 95 per cent alcohol. Thus any possible chance for change in the metabolic state between picking and killing was reduced to a minimum. In leaves of all three species it was noticed that at the end of 30 minutes chlorophyll was diffusing abundantly into the alcohol. Penetration and killing were therefore rapid. Keeping plant materials at ordinary temperatures and effecting a killing in 30 minutes must necessarily be fully as accurate as drying, if not more so. The method employed by DAVIS, DAISH, and SAWYER is unquestionably ideal, but since the material was gathered half a mile from the laboratory, it was impossible to adopt their procedure. They state that all their material was introduced into boiling alcohol not later than an hour after it was gathered. Thus by speeding up the routine in this work and killing the material in alcohol in 30 minutes, probably there is little difference in accuracy between the two methods. The samples were weighed to a tenth of a gram, and were preserved in alcohol, using a proportion

of about one part of sample to eight parts of alcohol by volume. The alcohol was previously treated with ammonia to render it slightly alkaline. It was tested after the sample had been killed in it and found to be practically neutral to litmus. The preserved plant materials were then stored in a cool place until they could be analyzed. At the time the samples were gathered, records of temperature and light intensity were made. These readings were taken every 10 minutes, and thus offer an accurate record of the fluctuations during the day. The instrument used in determining the intensity of light consisted of a thermometer with a blackened bulb inserted in a large glass tube from which all air was removed. Thus any rise in temperature would be due to the heat of the sun absorbed by the blackened bulb. These instruments were placed in the center of the plot and the data thus represent an average condition experienced by the plants. The temperature readings are reported in centigrade degrees, as are also those of light intensity.

#### ANALYSES

EXTRACTION.—The alcohol in which the material was killed and preserved was poured off and the leaves and petioles were thoroughly ground in a plate grinder. Fresh 80 per cent alcohol was added to the ground sample, which was then placed on a water bath and refluxed for two hours. The alcohol was then filtered off and the filtrate combined with the original alcohol. The residue was designated as *A* and the combined filtrates were then distilled off under reduced pressure between 40° and 50° C. When only a thick syrup remained it was taken up with water. Sometimes it was necessary to warm the water and to add a few pebbles in order to remove the oily, sticky mass which adhered to the walls of the distilling flask. The material was then clarified by treatment with Horne's dry lead in slight excess to precipitate all the chlorophyll and other fatty material. The coagulate was allowed to settle for a few minutes and removed. Enough of a saturated solution of disodium phosphate was added to precipitate the excess lead. The precipitate was then filtered off, made up to volume, and analyzed.

SIMPLE SUGARS AND SUCROSE.—Simple sugars were determined on 50 cc. aliquots of the clarified solution. The reducing power of all

sugar samples was obtained with the use of the SCHAFER-HARTMAN (32) modification of the MUNSON-WALKER method (16). In order to obtain the sucrose content of the solution, aliquots of the alcoholic extract were taken and hydrolyzed according to the chemical method as outlined in the Official Methods (16). The reducing power was again obtained and the sucrose content calculated from the difference in the reducing power before and after hydrolysis.

DEXTRINS AND SOLUBLE STARCHES.—The dextrins and soluble starches in a few preliminary experiments maintained such a uniform amount throughout the day and night that it was not considered wise to multiply analyses just for the sake of thoroughness. Thus these two groups are included with the insoluble starches.

INSOLUBLE STARCHES.—Residue *A* was transferred to a beaker with water and the mixture brought to boiling point in order to gelatinize the starch. The beaker was cooled to approximately 37° C., 10 cc. of fresh saliva added, and the whole mass allowed to digest overnight in an incubator heated to 37° C. Preliminary tests showed that most samples were entirely free from starch after two hours of digestion; but to insure complete hydrolysis of starch, and because the routine was so arranged, the samples were allowed to digest overnight. The material was filtered, the residue being designated as *B*. The filtrate was acidified to the extent of 2.5 per cent  $H_2SO_4$  and heated on a boiling water bath for 1.5 hours. After the solution was cooled, neutralized with concentrated NaOH, and turned slightly acid again, it was made up to volume and the reducing power was determined as before. Starch was reported as glucose.

HEMICELLULOSES.—Residue *B* was washed off the filter paper with 2.5 per cent sulphuric acid and heated on a boiling water bath for 2.5 hours. Great care was exercised in keeping all the water baths boiling uniformly, so that results might be comparable. The solution was filtered off, neutralized, turned acid again, analyzed as before, and reported as glucose.

All the results presented in this paper are reported in percentages of the green weight of the collected sample. CHIBNALL (7) favors the practice of reporting the analyses in terms of percentages of green weight, which he feels represent a truer record of conditions existing in the plant. DAVIS, DAISH, and SAWYER, however, state that re-

porting results on a dry weight basis does away with errors encountered because of the tendency of the leaves to be lower in moisture during the time when transpiration is excessive and higher when water loss is at a minimum. Without question, both these attitudes are reasonable, but although the latter does away with the uncontrollable factor of transpiration, it introduces a new error which is probably of greater dimension than the one eliminated. This error is due to the fact that great quantities of starch are deposited in leaves sufficient to make up as much as 10 per cent of their dry

TABLE I  
GRAMS OF GLUCOSE

| SAMPLE LEAVES    | SIMPLE SUGARS |        | SUCROSE |        | STARCH |        | HEMICELLULOSES |        |
|------------------|---------------|--------|---------|--------|--------|--------|----------------|--------|
|                  | A             | B      | A       | B      | A      | B      | A              | B      |
| Soy bean 23....  | 0.0213        | 0.0229 | 0.1084  | 0.1080 | 0.1225 | 0.1223 | 0.2261         | 0.2288 |
| Soy bean 19....  | 0.0346        | 0.0354 | 0.0425  | 0.0425 | 0.1678 | 0.1691 | 0.2317         | 0.2351 |
| Soy bean 16....  | 0.0345        | 0.0355 | 0.0731  | 0.0728 | 0.0886 | 0.0886 | 0.3330         | 0.3371 |
| Soy bean 20....  | 0.0338        | 0.0341 | 0.0829  | 0.0827 | 0.2964 | 0.2944 | 0.3733         | 0.3744 |
| Potato 17....    | 0.0353        | 0.0371 | 0.0890  | 0.0893 | 0.1608 | 0.1608 | 0.1286         | 0.1300 |
| Potato 19....    | 0.0277        | 0.0264 | 0.0339  | 0.0333 | 0.0513 | 0.0507 | 0.1397         | 0.1375 |
| Potato 18....    | 0.0464        | 0.0450 | 0.0846  | 0.0846 | 0.1438 | 0.1442 | 0.1760         | 0.1798 |
| Potato 10....    | 0.0433        | 0.0415 | 0.1208  | 0.1108 | 0.2360 | 0.2384 | 0.1675         | 0.1655 |
| Sunflower 20.... | 0.0732        | 0.0733 | 0.0347  | 0.0346 | 0.0580 | 0.0582 | 0.0776         | 0.0761 |
| Sunflower 13.... | 0.0830        | 0.0841 | 0.0983  | 0.0983 | 0.0987 | 0.0993 | 0.3046         | 0.3087 |
| Sunflower 12.... | 0.1057        | 0.1063 | 0.0560  | 0.0558 | 0.0782 | 0.0785 | 0.0920         | 0.0955 |
| Sunflower 17.... | 0.0853        | 0.0854 | 0.0459  | 0.0441 | 0.0448 | 0.0447 | 0.2699         | 0.2652 |

weight. Thus, when starch is reported in terms of dry weight it in itself makes up a considerable portion of that dry weight. Therefore, at such times the starch values will be too low. On the other hand, if it is reported in terms of green weight when transpiration is active, less water will be present and hence the values will be high. There is little preference between the two methods.

Since this paper embodies the results of some 4000 analyses, it was necessary to minimize the work as much as possible. When the first series of samples was gathered, duplicates were taken of each sample. Later, however, single samples were taken because it was found that two samples picked at the same time gave results which harmonized so well that it was not considered necessary to double the analyses merely to have the results as averages. Table I shows how closely the analyses compare for two samples picked at exactly the same time.

Although these duplicates were run on all the first series, only four collections of each plant are reported here. All the data of series 1, to be presented later, are averages of the duplicate samples, while the remaining series are based on the analysis of a single sample. Aliquots of the extracted samples were analyzed.

It is evident from table I that analyses of the simple sugars, sucrose, and starch are not widely variable. Greater variability is encountered in the hemicelluloses. This can be expected since the method of extraction is but relative and not absolute. This fact is kept in mind when the results are discussed.

#### CONDITIONS OF EXPERIMENT

It may be well to compare the environmental conditions of the six series. The samples for series 1, 2, and 2a were collected in 1926 on July 15-16, August 26-27, and September 15 respectively; series 3, 4, and 5 were collected in 1927 on July 7-8, August 11-12, and September 9-10 respectively. Series 1, 2, 3, and 4 represent complete series, that is, all three plants were used. Series 2a represents a special study in which only sunflowers were collected, but instead of collecting samples once each hour, they were gathered at 10-minute intervals. Series 5 includes studies of the sunflower and soy bean. Due to excessive drought the potatoes had died. The days on which series 1, 2, and 2a were collected were on the whole warmer than the days on which the later series were collected. During the day on which series 1 was collected there was very little air movement, and the sun was continually bright. The two dips in the light intensity curves (fig. 1) were due to passing clouds, of which there were but few. The night temperature remained around 11° C., although at one time it dropped to 8° C. Series 2 (fig. 1) was likewise collected under favorable conditions. Although there were more floating clouds than there had been during the earlier collection, the air was quiet and the temperature was higher both day and night than in series 1.

It will be noted that during the day on which series 2a was collected (fig. 3) the temperature curve as well as the light curve was nearly a straight line. Since this collection required only three hours, it was much easier to select a uniform period in which to gather the material.



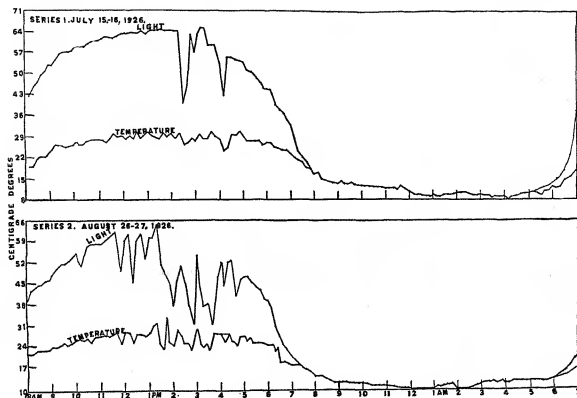


FIG. 1.—Light intensity and temperature records in centigrade degrees for series 1 and 2.

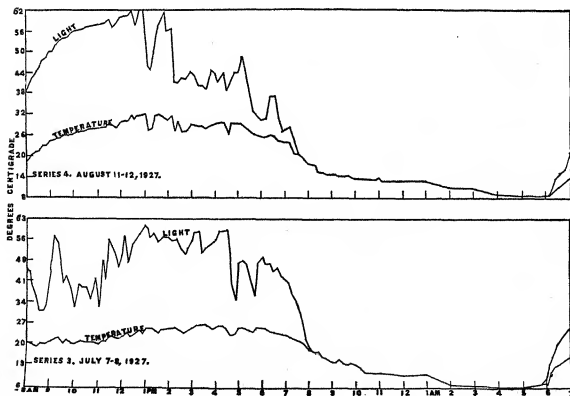


FIG. 2.—Light intensity and temperature records in centigrade degrees for series 3 and 4.

In 1927 the periods were not nearly so ideal as in the previous year. Series 3 was collected on a windy, cold day. The sky was almost entirely overcast, and the temperature was low all morning (fig. 2). The sun came out about 1:00 P.M., and although the temperature rose somewhat the wind was distinctly cold. The day on which series 4 was gathered was warmer and the sun was shining all

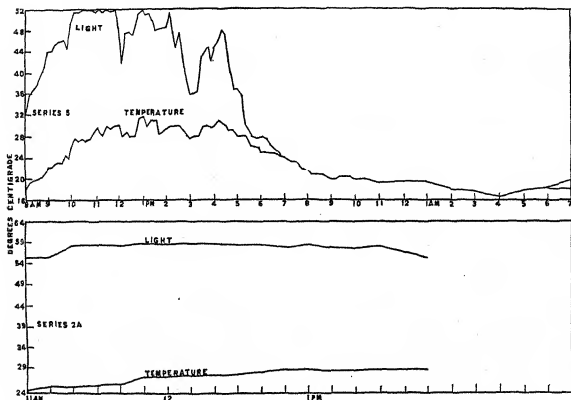


FIG. 3.—Light intensity and temperature records in centigrade degrees for series 2a and 5.

morning and intermittently during the afternoon. There was little or no wind, but the plants were experiencing a 3-weeks period of drought because of which the potatoes eventually died. Series 5 was collected under more favorable conditions, although there still was a lack of soil moisture (fig. 3). The sun shone all day with only an occasional cloud. The day and night were very warm, and the air was relatively quiet.

#### Discussion and conclusions

A summary of the foregoing data shows that they bear on several points of interest to the physiologist. A casual glance at the graphs

reveals the roughness of the curves, which is evidence that the periods of time between sampling were too long. Where only 10 minutes were allowed to elapse between consecutive samples, as in fig. 18, the curves representing the various forms of compounds are relatively smooth, thus better illustrating the actual carbohydrate changes going on within the leaf. Perhaps future studies of carbon assimilation would afford more accurate results if they were made from material gathered at very short intervals.

Perhaps, too, further experiments regarding carbon assimilation would be strengthened were the latitude at which the plants were grown taken into consideration. No one has attempted to show to what extent differences in latitude may affect the amount and kind of predominating sugars or carbohydrates found in leaves. GARNER and ALLARD (17), NIGHTINGALE (23), and others have shown that latitude will affect the flowering and reproduction of plants. CLEMENTS (8) has shown that different proportions of fertilizer salt combinations will result in distinct differences in the amount of carbohydrates present in the plants concerned, and that the effect a salt combination has on the quality of carbohydrates is in turn modified by different day lengths. It is therefore necessary to study considerable data gathered from the same kinds of plants grown under the same conditions, and then to compare these data with those obtained in a different latitude, other conditions remaining the same.

The analyses reported here indicate that sucrose is not the primary sugar of photosynthesis, and thus oppose the contentions of PARKIN, BROWN and MORRIS, and DAVIS, DAISH, and SAWYER. Perhaps the assumption that glucose and fructose are the only hexose sugars generally found in the leaves of plants led them to that conclusion, since sucrose is composed of these two forms. Were glucose the first sugar formed by the plant, a change of part of it to fructose would be necessary before the sucrose could possibly be formed. The well-known enol conversion of glucose to fructose and mannose is taken by these workers as an all important factor. The fact that mannose does not appear in leaves, of course, indicates that this conversion does not take place; hence the conclusion that fructose and glucose arise from sucrose which therefore must be the first sugar synthesized. Such reasoning does not appear necessary to eliminate the

possibility of the existence of an enolic form of glucose, since this form exists only in an alkaline solution, while the changes taking place in the leaf are going on under acid conditions.

The second argument, that sucrose is the first product of synthesis because sucrose is more abundant in the leaves of plants during the day than are simple sugars, is not borne out by the plants

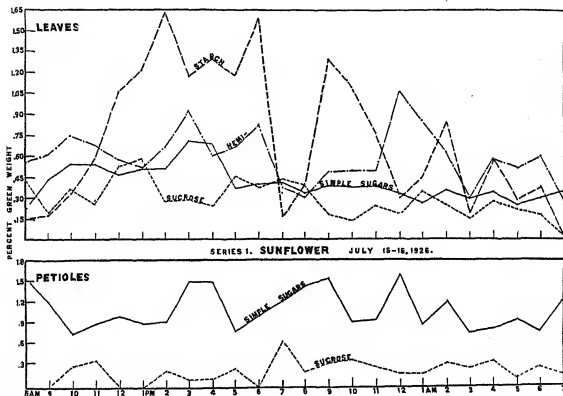


FIG. 4.—Hourly variations in content of various carbohydrates in leaves and petioles of sunflower gathered July 15-16, 1926 (series 1).

studied. In series 1 of all three plants (figs. 4-6), sucrose is relatively high, but in the remainder of the series the simple sugars are always higher than the disaccharides.

The third argument, favoring sucrose as the primary sugar, is that its abundance in leaves varies more than that of the simple sugars, and hence it is the primary sugar. STANESCU (33) even classifies plants on that basis. He divides them into the saccharophiles, those plants in the leaves of which soluble carbohydrates vary greatly in content; the typical amylophiles in which the content of the polysaccharides varies more than that of the simpler carbohydrates;

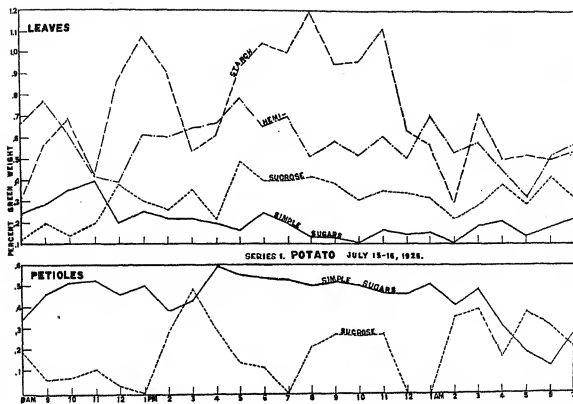


FIG. 5.—Hourly variations in content of various carbohydrates in leaves and petioles of potato gathered July 15-16, 1926 (series 1).

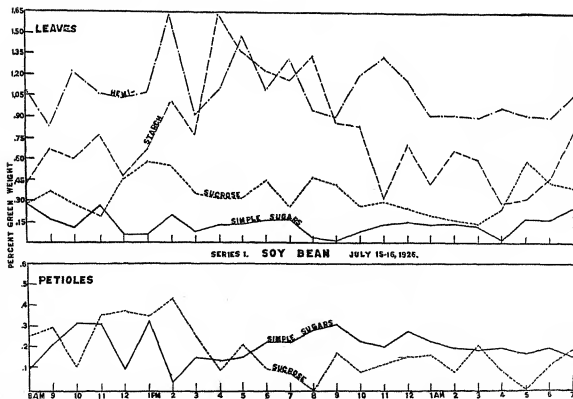


FIG. 6.—Hourly variations in content of various carbohydrates in leaves and petioles of soy bean gathered July 15-16, 1926 (series 1).

and the amylophiles in which the variations in the content of either the simple or complex carbohydrates are not outstanding. If such groupings were possible, the results presented here would show that according to series 1, all three species of plants were saccharophiles; whereas the first collection of samples from the same kinds of plants the following year (figs. 7-9) would indicate that they were typical

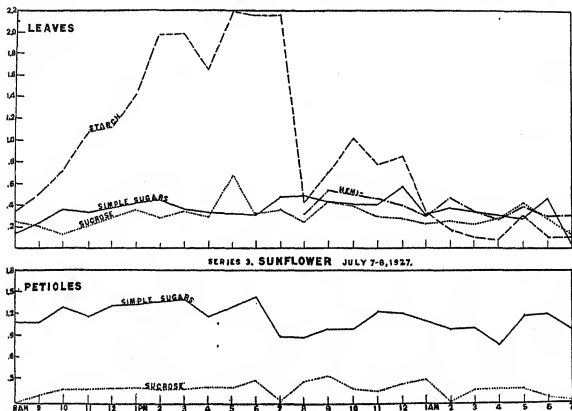


FIG. 7.—Hourly variations in content of various carbohydrates in leaves and petioles of sunflower gathered July 7-8, 1927 (series 3).

amylophiles. In other words, it seems unwise to use the behavior of carbohydrates in leaves as a basis for the classification of plants, since under varying conditions of environment the plant will respond differently. Thus at times the curves for starches and hemicelluloses vary more than those of the simple forms. These facts indicate that sucrose does not always vary more than simple sugars.

In a number of instances the data show no sucrose at all in the leaves until long after photosynthesis has begun. Thus in series 2 (fig. 10), sucrose disappears from the sunflower leaves at 5:00 A.M. and does not reappear before 7:00 A.M. In the same series (fig. 11) the potato leaves are entirely free from sucrose between 6:00 and



7:00 A.M. In series 3 (fig. 8), of the potato, sucrose disappears at 4:00 A.M. and does not appear before 7:00 A.M. In series 4 (fig. 12) it disappears at 1:00 A.M. In series 2 and 4 of the soy beans (figs. 13, 14) sucrose likewise disappears. Is it not significant that in series 2 (figs. 10, 11, 13) all three species used were free from sucrose during the morning hours? These facts, together with the microchemical

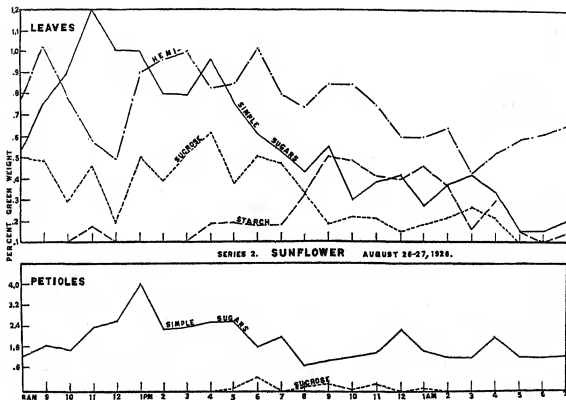


FIG. 10.—Hourly variations in content of various carbohydrates in leaves and petioles of sunflower gathered August 26-27, 1926 (series 2).

observations of STRAKOSCH (35) that simple sugars alone appear in the mesophyll cells, seem to point decidedly in favor of simple sugars and not sucrose as the primary sugar of photosynthesis. It is entirely possible that pentoses and hexoses both are among the first-formed sugars.

A somewhat unusual concept regarding the rôle of sucrose is that offered by PRIESTLEY, who holds that it is not concerned with photosynthesis at all but that it is found as a by-product of the aging protoplasm of cells, and as these cells become vacuolated sucrose is released. PRIESTLEY advances this theory from a chemical view-



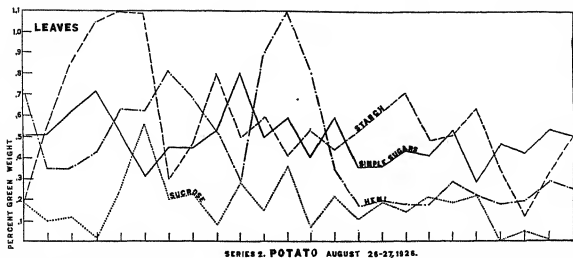


FIG. 11.—Hourly variations in content of various carbohydrates in leaves and petioles of potato gathered August 26-27, 1926 (series 2).

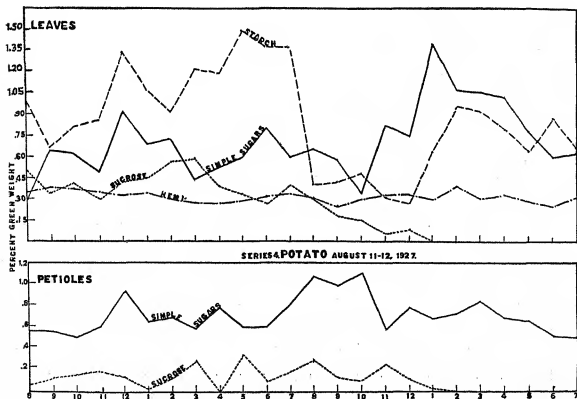


FIG. 12.—Hourly variations in content of various carbohydrates in leaves and petioles of potato gathered August 11-12, 1927 (series 4).

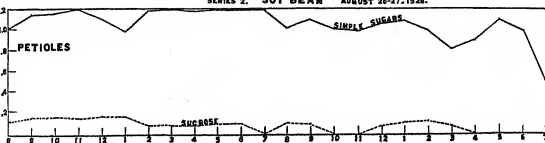
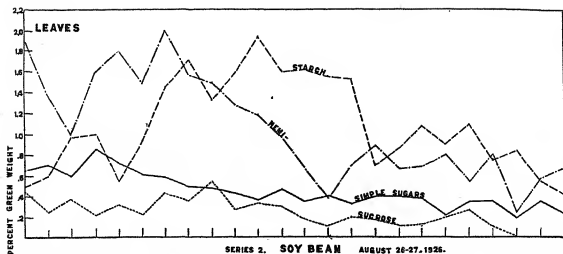


FIG. 13.—Hourly variations in content of various carbohydrates in leaves and petioles of soy bean gathered August 26-27, 1926 (series 2).

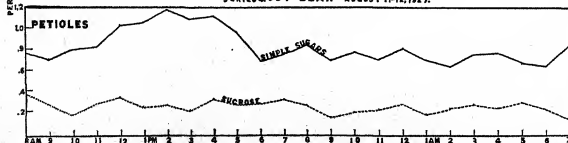
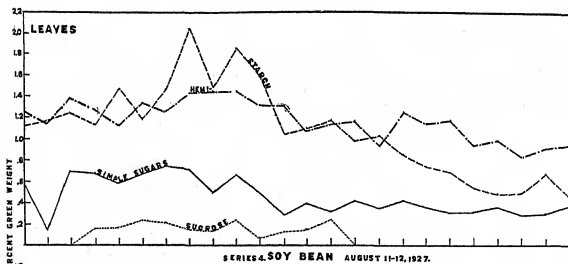


FIG. 14.—Hourly changes in content of various carbohydrates in leaves and petioles of soy bean gathered August 11-12, 1927 (series 4).

point. The fact that sucrose has never been synthesized in the test-tube seems to be an important one. If this were to be accepted as convincing evidence, it would indeed be difficult to account for many of the compounds existing in plants. PRIESTLEY states:

All these considerations have led the present writer to this conclusion, that the cane sugar widespread in plant tissues but not concentrated in amount as a storage product arises as a secondary product, formed first of all during the complicated metabolism associated with protoplasmic construction and therefore present in the growing cell.

Apparently where sucrose appears in growing points or anywhere else in small amounts, it arises katabolically. This of course eliminates the places of storage, as in the sugar beet or cane, and must also eliminate the leaves, for there is considerable sucrose stored there. Since these exceptions are taken, it may be well to ask how the sucrose was formed in these cases.

A great part of the data PRIESTLEY used in arriving at his conclusions was gathered from roots and stems. The present studies furnish evidence which indicates that sucrose can be formed from the simple sugars. Using the three species of plants already mentioned, studies were based on the weekly changes of the nitrogenous compounds, carbohydrates, and fats. In the roots of the soy bean sucrose was found to be entirely absent until late in the season (August 1926), at which time the simple sugars were very highly concentrated: then sucrose appeared. In the potato stems sucrose was present in small quantities during early July, and then was absent until the simple sugars reached an amount equivalent to 8 per cent of the dry weight, when it reappeared and increased in amount as the simple sugars decreased. In 1927 this occurred even more strikingly. As the simple sugars increased in quantity the sucrose curve rose, and a week later fats were accumulating along with the sucrose apparently at the expense of the simple sugars. In the stems of the sunflower, sucrose was insignificant in amount until the middle of August. Up to that time the simple sugars accumulated, until they constituted 20 per cent of the dry material; then the content of sucrose rose from less than 1 to 15 per cent of the dry weight, while at the same time the simple sugars decreased.

These data, many more of which could be cited, indicate several

things: first, sucrose was not present in the roots of the soy bean and stems of the potato during the active growing season of the years mentioned. The single fact that sucrose will appear one year in a certain plant part and not the next is sufficient evidence that its origin cannot be such as PRIESTLEY maintains. Second, sucrose appeared abundantly only after the simple sugars reached a certain maximum. To say that sucrose gave rise to simple sugars seems less likely than the reverse situation. Further, after sucrose appears in regions where simple sugars are abundant, fats also appear. Thus it would seem that fats and sucrose here were formed from the same compounds, simple sugars. If this is the case, it would seem probable likewise that the same situation exists in the leaves, and that sucrose is not the primary form there but is derived from some of the primary forms.

The data presented indicate that the simple sugars are the usual forms in which carbohydrates move from one part of the plant to another. All the graphs, however, show that sucrose is present in the petioles at some time during each series. It is usually relatively more abundant during the early part of the growing season than later. Since this is also true in the leaves, it would appear that sucrose moves into the petioles as such, and is not (as DAVIS, DAISH, and SAWYER suggest) inverted to form simple sugars on entering the veins. In only one case (series 4, soy bean) was sucrose present in the petioles when the leaves were free from it. Seemingly it disappears from the petioles about the same time as it does from the leaves. Thus it is apparent that the simple sugars are the common forms in which carbohydrates are moved. Sucrose, too, appears as a petiolar sugar but it is not nearly so abundant as the simple sugars, nor is it found in the petiole at all times.

These studies show the inadequacy of present-day explanations of the processes concerned in translocation, and although they do not furnish a basis for the development of a new concept, it will not be amiss, perhaps, to point out the demands which must be satisfied by any theory explaining the movement of food through plant tissues. DIXON (15) argues that because dyes will move downward through the xylem, synthesized foods move through the same tissue. MASON and MASKELL (19), however, show that by girdling a stem

to the xylem the downward movement of food will be prevented but not that of the dye. This finding, together with the results of CURTIS (9-11), demonstrates that translocation of food materials takes place in phloem tissue. That foods do not move through these tissues by simple diffusion is apparently shown by the behavior of the carbohydrates in the leaves of the sunflower. The following data indicate that carbohydrates move out of the leaves and petioles in such large quantities and so rapidly that an explanation based on the principles of diffusion is inadequate. The starch in the leaves of the sunflower in series 1, 3, 4, and 5 drops from the maximum amount to the minimum in the comparatively short time of one or two hours. In series 1, therefore, the starch content of the sunflower leaves decreases from 1.6 to 0.2 per cent in one hour. Were this amount reported on a dry weight basis it would be equivalent to approximately 9 per cent of the dry material of the leaf. An explanation of translocation, therefore, must not only account for the rapidity of food movement which is demonstrated by these data, but it must also account for the movement of large quantities of material.

A third requirement of a theory explaining translocation is perhaps demonstrated by the behavior of the starch curve of the sunflower leaves in series 1 after sunset. In series 1, the starch content of the leaves increases from approximately 0.4 to 1.2 per cent. This increase apparently cannot be explained by assuming that other carbohydrates are being changed to starch since these forms remain practically constant. Since photosynthesis is no longer going on, it is probable that the sugar from which this starch is formed is really coming back into the leaf from the petiole. BENNETT (3), using a mosaic virus as an indicator of translocation, shows that food movements can be reversed. Thus when the young first year canes of the raspberry are growing vigorously, the movement of foods is apparently downward toward the crown of the plant. When the top of such a cane is pruned off and all its leaves removed, however, the virus which seems closely associated with the food stream goes back up the stem. Thus the third requirement of a theory explaining translocation demands recognition of reversibility of the food streams and the rapidity with which this phenomenon is accomplished.

The data representing the variation in the content of hemicelluloses indicate that the part played by these reserves in the metabolism of plants has been underestimated by physiologists generally. NIGHTINGALE summarizes the consensus of opinion regarding the activity of hemicelluloses by suggesting that they are a type of reserve which can be used by the plant as a source of energy when other foods are practically consumed. During the summer of 1926, the hemicelluloses varied considerably each day, and it should be remembered that climatic conditions favored carbohydrate accumulation.

The fluctuations of the hemicelluloses indicate that they are important food reserves. In series 1 this group of reserves does not vary so greatly, but in the night after the second drop of the starch curve the hemicelluloses increase considerably, and as they again disappear more starch appears. The petioles contain constant amounts of these polysaccharides. Likewise in the other series there are instances which show that simple sugars, sucrose, or starch may increase in quantity while the hemicelluloses decrease, and vice versa.

There are many variations in the quantities of hemicelluloses in the leaves that are rather large and suddenly abrupt (figs. 15-17). At first they may be thought to be due to analytical inexactness. To test this, series 2a was collected. Collections were made for 3 hours at intervals of 10 minutes (fig. 18). In only a few cases is the variation within a 10-minute period slightly over 0.1 per cent. The curve for starch varies but slightly. The curve representing the hemicelluloses offers a basis for a new concept regarding these seemingly inactive reserves. Dropping from 0.72 at 11:40 A.M. to 0.32 per cent at 12:30 P.M., it shows a small decrease at each 10-minute interval. It rises again to 0.8 per cent during the next 20-minute period. Thus it appears that any large fluctuation which occurs in the space of an hour might well be considered an accurate record, and not just a haphazard scattering of points due to inadequate analyses.

These facts seem to indicate that when the formation of carbohydrates is taking place rapidly, the hemicelluloses behave in precisely the same way as starch, but when starch forms rapidly enough to remove the sugars from the chloroplastid solution the latter do not diffuse in large quantities into the vacuole of the cells

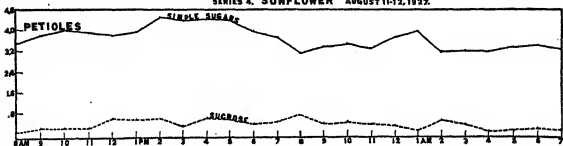
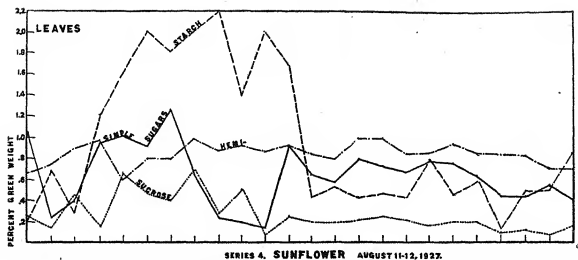


FIG. 15.—Hourly variations in content of various carbohydrates in leaves and petioles of sunflower gathered August 11-12, 1927 (series 4).

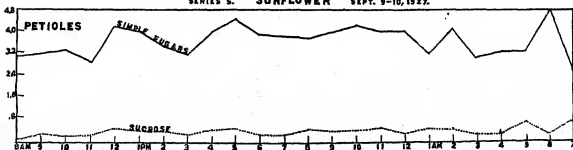
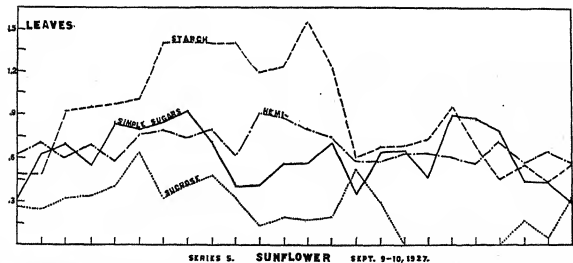


FIG. 16.—Hourly variations in content of various carbohydrates in leaves and petioles of sunflower gathered September 9-10, 1927 (series 5).

where sucrose apparently is formed. When sucrose forms abundantly, it is evidence for the conclusion that the simple sugars are diffusing into the vacuole more rapidly than they can be utilized there for energy, or translocated to the other parts of the plant. But when the content of hemicelluloses increases rapidly, the vacuolar sugars may be so concentrated as to favor the formation of some reserve which is entirely removed from the solution.

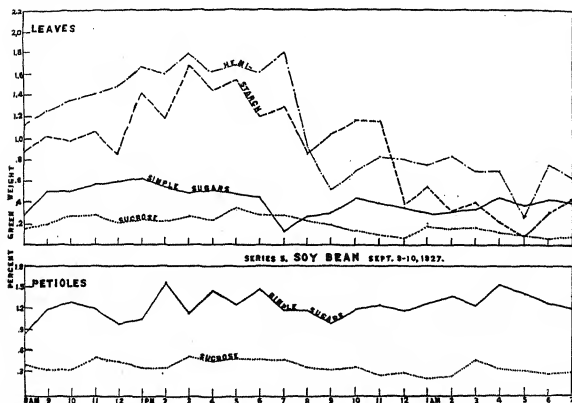


FIG. 17.—Hourly changes in content of various carbohydrates in leaves and petioles of soy bean gathered September 9-10, 1927 (series 5).

Large and rapid variations in the content of hemicelluloses apparently are of entirely different physiological significance from a maintenance of a definite reserve supply throughout a day. Thus during the first summer, the climatic conditions of temperature and rainfall were such that the plants produced rapid vegetative growth and fruited heavily. During the second year, however, rainfall was at times so deficient that the plants wilted during the day. This condition, together with the lower temperature, was not conducive to such an excellent crop as was obtained the previous year. During the



first year the changes in variations in the amounts of hemicelluloses were not only large but also rapid. Here this group of compounds seemed apparently a reserve only; but during the next year, when moisture was wanting, the hemicelluloses seemed to act as holders of water (figs. 8, 12, 14-18).

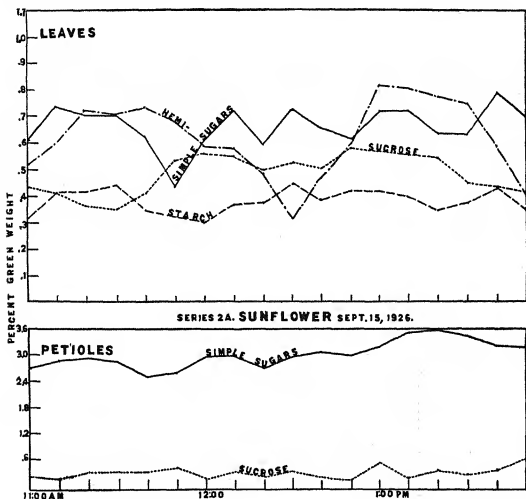


FIG. 18.—Variations in content of various carbohydrates in leaves and petioles of sunflower gathered at 10-minute intervals September 15, 1927 (series 2a).

The material for these analyses (table II) was gathered at 1:00 A.M. on the dates indicated. During this year the plants did not suffer from a lack of moisture or heat, and the behavior of the hemicellulose content was much the same in all three plants. Table III is directly comparable with table II but represents the results secured in 1927.

From table III it is evident that the soy bean maintained the

largest amount of hemicelluloses during the first four weeks of the study, when the temperature was relatively low, and maintained a higher quantity during the last seven weeks, during which time the plants received only 0.1 inch of rainfall. These plants wilted during

TABLE II  
HEMICELLULOSE CONTENT OF LEAVES, 1926

|                | PERCENTAGE DRY WEIGHT |         |         |         |        |         |         |         |         |          |          |
|----------------|-----------------------|---------|---------|---------|--------|---------|---------|---------|---------|----------|----------|
|                | July 5                | July 11 | July 18 | July 26 | Aug. 9 | Aug. 16 | Aug. 22 | Aug. 29 | Sept. 5 | Sept. 12 | Sept. 19 |
| Soy bean.....  | 4.7                   | 3.1     | 3.2     | 3.3     | 3.3    | 3.3     | 2.7     | 2.8     | 4.1     | 3.6      | 4.4      |
| Sunflower..... | 3.3                   | 2.8     | 4.3     | 2.7     | 2.8    | 2.1     | 2.8     | 3.4     | 3.9     | 3.9      | 4.0      |
| Potato.....    | 3.7                   | 2.7     | 3.0     | 3.4     | 3.4    | 2.6     | 3.4     | 3.9     | 3.6     | 2.0      | 3.1      |

the early days of the drought but later remained turgid throughout the day, indicating that because of the increased content of these water-holding compounds they were able to resist desiccation.

The sunflower was intermediate between the potato and the soy bean in drought resistance. It wilted each day and at first recovered

TABLE III  
HEMICELLULOSE CONTENT OF LEAVES, 1927

|                | PERCENTAGE DRY WEIGHT |         |         |         |        |         |         |         |         |          |          |
|----------------|-----------------------|---------|---------|---------|--------|---------|---------|---------|---------|----------|----------|
|                | July 5                | July 11 | July 18 | July 26 | Aug. 9 | Aug. 16 | Aug. 22 | Aug. 29 | Sept. 5 | Sept. 12 | Sept. 19 |
| Soy bean.....  | 5.1                   | 3.6     | 4.2     | 4.0     | 4.8    | 4.1     | 5.4     | 6.5     | 6.0     | 4.1      | 3.2      |
| Sunflower..... | 2.9                   | 3.0     | 2.2     | 3.0     | 3.9    | 2.5     | 3.0     | 2.4     | 2.8     | 2.3      | 1.9      |
| Potato.....    | 2.4                   | 2.4     | 2.2     | 2.0     | 3.1    | 2.3     | 2.2     | 2.1     | 2.2     | 1.5      | ....     |

during the night, but during the last week it remained in part permanently wilted. Its content of hemicelluloses is greatly less than that of the soy bean. The potato dried up completely.

Thus apparently the presence of large amounts of hemicelluloses is associated with drought resistance. Some attempts have been made by various workers to account for this resistance of plants by their content of pentosans. It seems, however, that the hemicelluloses as a group must be considered as possessing that property,

since gums and pectic substances are fully as hygroscopic as the pentosans.

From these data and observations, it is clear that the rôle of hemicelluloses is not a single one, for under favorable conditions for rapid carbohydrate formation they seem to act as temporary reserves in the leaves; while under drought conditions their accumulation and maintenance in the leaves add to the drought resistance of the plant. In addition to these two rôles, these reserves as found in stems and roots of perennial plants not only contribute to the strength of tissues as wall thickenings, but also serve as energy reserves.

### Summary

1. Data on carbohydrate metabolism are presented which were gathered from the potato, soy bean, and sunflower during the summers of 1926 and 1927.
2. Distinct differences in rapidity of formation and translocation of carbohydrates were observed in plants grown under the favorable environmental conditions of 1926 and the unfavorable conditions of 1927.
3. Sucrose apparently is not the first sugar formed by leaves, nor is it formed as a by-product of vacuolating protoplasm. Its function in the leaf seems to be that of temporary storage.
4. The simple sugars (hexoses and pentoses) seem to be the first sugars synthesized by plants.
5. From the data, observations are made which impose certain requirements upon any theory which attempts to explain the translocation of food materials from the leaves.
6. Variations in the content of hemicelluloses are not only large but frequent in leaves growing in a favorable environment. Under such conditions these materials apparently serve as temporary reserves.
7. When growing conditions are unfavorable, due to deficient moisture or warmth, the amount of hemicelluloses is relatively large but fluctuations are not observed.
8. A plant which is capable of producing and maintaining a large content of hemicelluloses in its leaves apparently can resist drought.

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#### LITERATURE CITED

1. ALEXANDROV, W. G., Über tägliche Veränderungen des Starkegehaltes in Blättern. Ber. Deutsch. Bot. Ges. 44:217-226. 1926.
2. BAEYER, A., Über die Wasserentziehung und ihre Bedeutung für das Pflanzenleben und die Gährung. 3:63-75. 1870.
3. BENNETT, C. W., The virus diseases of the raspberry. Mich. State College Tech. Bull. 80. 1-38. 1927.
4. BROWN, H. T., and MORRIS, G. H., A contribution to the chemistry and physiology of foliage leaves. Jour. Chem. Soc. 63:604-677. 1893.
5. BOEHM, J. A., Beiträge zur näheren Kenntnis des Chlorophylls. Sitzber. K. Akad. Wiss. Wien. 22:479-487. 1856.
6. CAMPBELL, A. V., Carbohydrates of the mangold leaf. Jour. Agric. Sci. 4: 248-259. 1911.
7. CHIBNALL, A. C., Diurnal variations in the total nitrogen content of foliage leaves. Ann. Botany 37:511-518. 1923.
8. CLEMENTS, H. F., Plant nutrition studies in relation to the triangular system of water cultures. Plant Physiol. 3:441-458. 1928.
9. CURTIS, O. F., The upward translocation of foods in woody plants. I. Tissues concerned in translocation. Amer. Jour. Bot. 7:101-124. 1920.
10. ———, The transport of foods and nutrients in woody plants. Abs. Brit. Assoc. Adv. Sci. Rpt. 92:443-444. 1924.
11. ———, Studies on the tissues concerned in the transfer of solutes in plants. The effect on the upward transfer of solutes of cutting the xylem as compared with that of cutting the phloem. Ann. Botany 39:573-585. 1925.
12. DAVIS, W. A., DAISH, A. J., and SAWYER, G. C., Studies of the formation and translocation of carbohydrates in plants. I. The carbohydrates of the mangold leaf. Jour. Agric. Sci. 7:255-326. 1916.
13. DAVIS, W. A., and SAWYER, G. C., Studies of the formation and translocation of carbohydrates in plants. III. The carbohydrates of the leaf and leaf stalks of the potato. The mechanism of the degradation of starch in the leaf. Jour. Agric. Sci. 7:352-384. 1916.

14. DELEANO, N. T., Untersuchungen über die in Weinblättern enthaltenen Kohlenhydrate und stickstoffhaltigen Körper. *Zeit. Physiol. Chem.* 80: 79-94. 1912.
15. DIXON, H. H., Transport of organic substances in plants. *Nature (London)* 110: 547-551. 1922.
16. DOOLITTLE, et al. (editing committee), Official and tentative methods of analysis of the Association of Official Agricultural Chemists. 2d ed. pp. xvi+535. Assoc. Off. Agric. Chem. Wash. D.C. 1925.
17. GARNER, W. W., and ALLARD, H. A., Further studies in photoperiodism: the response of the plant to relative length of day and night. *Jour. Agric. Res.* 23: 871-920. 1923.
18. LANGNER, W., Über die Verteilung der Stärke in Laubblättern zur verschiedenen Tageszeiten. *Jahrb. Wiss. Bot.* 17: 291-333. 1927.
19. MASON, T. G., and MASKELL, E. J., Studies on the transport of carbohydrates in the cotton plant. I. A study of diurnal variation in the carbohydrates of leaf, bark, and wood, and of the effects of ringing. *Ann. Botany* 42: 189-253. 1928.
20. MEYER, A., Über die Assimilations producte der Laubblätter angiospermer Pflanzen. *Bot. Zeit.* 43: 27-32. 1885.
21. MILLER, E. C., Daily variation of the carbohydrates in the leaves of corn and the sorghums. *Jour. Agric. Res.* 27: 785-808. 1924.
22. VON MOHL, H., Untersuchungen über die anatomischen Verhältnisse des Chlorophylls. *Diss.* 1837. (Cited from BROWN and MORRIS.)
23. NIGHTINGALE, G. T., The chemical composition of plants in relation to photoperiodic changes. *Wis. Exp. Sta. Res. Bull.* 74. 1-68. 1927.
24. PARKIN, J., The carbohydrates of the foliage leaf of the snowdrop (*Galanthus nivalis*) and their bearing on the first sugar of synthesis. *Biochem. Jour.* 6: 1-47. 1912.
25. PELLET, M., *Bull. Assoc. Chim. Sucre* 31: 173. (Cited from DAVIS, DAISH, and SAWYER.)
26. PRIESTLEY, J. H., The first sugar of photosynthesis and the rôle of cane sugar in the plant. *New Phytol.* 23: 255-265. 1924.
27. ROBERTSON, R. A., IRVINE, J. C., and DOBSON, M. E., A polarimetric study of sucroclastic enzymes in *Beta vulgaris*. *Biochem. Jour.* 4: 258-273. 1909.
28. RUHLAND, J., Untersuchungen über den Kohlenhydratstoffwechsel von *Beta vulgaris* (Zuck.). *Jahrb. Wiss. Bot.* 50: 200-373. 1911. (Cited from BROWN and MORRIS.)
29. SACHS, J., Über den Einfluss des Lichtes auf die Bildung des Amylums in den Chlorophyllkörnern. *Bot. Zeit.* 20: 365. 1862.
30. ———, Über die Auflösung und Neubildung des Amylums in den Chlorophyllkörnern bei wechselnder Beleuchtung. *Bot. Zeit.* 22: 289-294. 1864.
31. SCHIMPER, A. F. W., Untersuchungen über die Entstehung der Stärkekörner. *Bot. Zeit.* 38: 881-902. 1880.

32. SHAFFER, E. A., and HARTMAN, A. F., The iodometric determination of copper and its use in sugar analysis. Jour. Biol. Chem. 45:365-390. 1920.
33. STANESCU, P. P., Les variations quantitatives des substances hydrocarbonées dans les feuilles des plantes vertes au cours d'une journée. Compt. Rend. Acad. Sci. 182:154-156. 1926.
34. STEPHANI, F., Untersuchungen über reduzierenden und nicht reduzierenden Zucker in den Beta-Rüben während des Wachstums und der Lagerung. Kühn-Archiv. 1:107. 1911. (Cited from DAVIS, DAISH, and SAWYER.)
35. STRAKOSCH, S., Ein Beitrag zur Kenntnis des Kohlenhydratstoffwechsels von *Beta vulgaris* (Zuck.). Sitzber. K. Akad. Wiss. Wien. 116:855-869. 1907.
36. TOTTINGHAM, W. E., LEPKOVSKY, S., SCHULZ, E. R., and LINK, K. P., Climatic effects in the metabolism of the sugar beet. Jour. Agric. Res. 33: 59-76. 1926.

CHEMICAL CONDITIONS IN MATURATION,  
DORMANCY, AND GERMINATION OF  
SEEDS OF GYMNOCLADUS DIOICA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 402

GEORGE J. RALEIGH

(WITH SEVEN FIGURES)

Introduction

In the great group of plants with dormant seeds, the dormancy of which is occasioned by a seed coat which excludes water, may be included *Gymnocladus dioica*. Impermeable seed coats have been the subject of considerable botanical discussion. Most of the early work was concerned primarily with the anatomy of the seed, and with but passing attention to the physical and chemical features of water exclusion. PAMMEL (17) studied the anatomy of leguminous seeds, including *G. dioica*, and listed an extensive bibliography of the earlier work. EWART (7) made an extensive investigation of the longevity of seeds, and secured data on many seeds with hard coats. OHGA (15) recently discussed the longevity, water absorption, and life activity of the seeds of the lotus.

There have been many conflicting ideas as to what features of the hard seed coat give the water-excluding property to the seed. Most investigators are agreed that the Malpighian layer is responsible, but there is marked diversity of opinion as to what property of this layer is involved. BERGTHEIL and DAY (2), working with *Indigofera arrecta*, concluded that impermeability was due to a very thin outer layer of substance impermeable to water, but they were unable to ascertain with certainty the chemical nature of this layer. Two recent workers (3, 12) have attributed water exclusion to a rather narrow zone of the Malpighian layer, the so-called light line, which they consider to have different physical and chemical properties from the remainder. COE and MARTIN (3) noted the presence of pectic materials in the layer as indicated by microchemical tests.

NELSON (14) in a preliminary report suggested that water ex-

clusion might be brought about by deposit on the seed coats of mucilaginous inner pod material.

The economic aspect of "hard" seeds is of importance. A great number of our most useful plants produce some impervious seeds. The increasing rôle played by the clovers, alfalfa, and other legumes in agriculture has stimulated work on the causes of "hardness" in seeds, and on the practicable methods of overcoming the dormancy caused by water exclusion. In some economic legumes it has long been recognized that climatic conditions are responsible for varying percentages of impervious seeds. Dry ripening seasons tend to increase the percentage of seeds which do not imbibe water. When the number of impervious seeds is proportionally high, scarification of the seeds is profitable. In one of the methods found practicable, the seeds are blown against a roughened surface, cutting the impervious layers slightly and making the seeds pervious to water. With large seeds, however, such methods of scarification are not practicable, since the impervious layer is too heavy to be cut by such methods. In these cases treatment with strong sulphuric acid is found useful.

The writer has been interested in the seeds of *Gymnocladus dioica* for a number of years, and early in 1926 began work with the idea of obtaining data on the chemical changes in the seeds during the later stages of maturity and during germination, with special attention to the Malpighian layer of the seed coat. Much work has been done on the changes in fats, water-insoluble carbohydrates, and water-soluble carbohydrates in oil-containing seeds during development and germination, but it was thought that this developmental work could conveniently be carried along with the developmental study of the impermeable seed coat, and might yield data of value, some of which possibly would shed light on the major portion of the problem. On account of the possible rôle of the inner carpellary material of the pod in forming the water-excluding layer, it was considered best to ascertain some of the chemical changes in that area during the stages of later maturity.

But little work of an analytical nature has been done with *Gymnocladus*. BARKENBUS and ZIMMERMAN (1) have recently published a preliminary analysis of the mature seeds and pod, with special em-



phasis on the properties of the oil. Earlier work (11, 13) was done from the standpoint of the pharmacologist.

It was also considered advisable to determine if possible by experimental means the ways of effecting a change in the seed coat during development, as well as means of overcoming the water-excluding property of the naturally matured seed coats.

From the outset it was recognized that the data obtained on *Gymnocladus dioica* could not be expected to hold for the smaller seeds of economic importance, not even for those leguminous seeds already mentioned. It was hoped, however, that this large seed might yield some facts which would aid in diagnosing the cause of impermeability in those species of economic importance.

#### Materials and methods

It was not possible to study the development of seeds from the same source during successive years. The original lot of seeds came from a commercial seed company; seeds of the crop of 1926 were obtained from Manhattan, Kansas; while the work done in 1927 was carried on in part in the vicinity of Lafayette, Indiana. Analysis of the mature seeds of the three lots, however, showed but slight differences.

For the developmental study, material was gathered for analysis at various periods during the later stages of growth. Pods were obtained by knocking them from the tree, and an attempt was made to take pods at random so as to make the samples representative of the degree of maturity at that stage.

The immature pod of *Gymnocladus dioica* (exclusive of the contained seeds) can be separated easily into the hard outer portion, or pod proper, and the inner portion, which in its more immature stages is cellular and quite firm. This inner portion was analyzed at several periods during the late stages of development, and it was found that the seed could be divided easily into four portions. The outer part of the coat, or Malpighian layer, could be scraped off with a dull knife, the rupture occurring in the region of the osteosclerid cells. The endosperm could then be pulled from the inner portion of the coat, leaving the large portion still intact. This remainder was largely the

so-called nutrient layer. The rest of the seed consisted of the cotyledons and embryo. Samples of these various parts were preserved for analysis. An attempt was made during the late stages of maturity to correlate water loss with shrinking and the change from permeable to impermeable seeds.

Analysis of mature seeds was not run until the seeds had reached a low water content, and had become impermeable to water. The seeds were filed until the Malpighian layer was severed, and then placed in distilled water and allowed to absorb a maximum water content. After this absorption the seed could be separated into the parts described for the immature seed. This method of separating is subject to criticism, but it was thought to be the most reliable way of obtaining a good segregation of the parts of this very hard seed coat. Analysis of the water in which the seeds were soaked showed that the amount of sugar it contained was not large.

Methods of trial and error were used on the impermeable mature seed coat, and included such lines of attack as cold treatment, long continued soaking in water, acid treatment, exposure to violent changes in temperature, and the action of fungous growths.

For the work on the germinating stages, mature impermeable seeds were filed and soaked as described, and then placed in the germinator between blotting paper at a constant temperature of 25° C. Samples for analysis were taken at intervals during the growth of the young seedlings up to the end of the eighteenth day. In this work the cotyledons (and embryos) were analyzed.

#### CHEMISTRY

The methods of analysis were primarily those of KOCH (9). Samples were taken when possible from large well mixed lots. In some cases lack of material made proper sampling impossible; in practically all cases duplicate samples were analyzed. Samples when taken were placed in hot 95 per cent alcohol, with sufficient added to make the solution at least 70 per cent alcohol. Then the samples were kept at ice-box temperature, and analyses run as soon as convenient.

The procedure in this work consisted in separating the sample into three fractions: the ether-soluble, the alcohol-water-soluble, and the

insoluble (insoluble in ether, alcohol, and water) fractions. The ether-soluble fraction was largely fat, but contained some phospholipins. Reducing and non-reducing sugars were run by the Bertrand volumetric method. The slow invertase method was used in determining sucrose. In testing quantitatively for starch and hemicellulose, hydrolysis was brought about by boiling the sample for three hours in 6 per cent HCl. The acidity of this solution was slightly higher than that now recommended, but the first samples were run with this dilution, and it was thought best to continue the same program to insure comparable results, rather than change to the more recently recommended procedure.

Total pectic materials were determined by the methods suggested by CONRAD (4), slightly modified. Samples to be analyzed were slowly dried to constant weight, ground in a mortar, and extracted with 250 cc. N/30 HCl at boiling temperature for one hour. The soluble portion was filtered off, the residue washed and dried, reground in a mortar, and the process repeated. This procedure was followed three times. In the fourth and last extraction the procedure was the same except that 1 per cent ammonium citrate was used instead of N/30 HCl.

Microchemical tests on the finely ground dry residue following the fourth extraction showed but slight traces of pectic materials. Quantitative tests were made on the Malpighian layer cut from green seeds, and on the layer after soaking it from the mature seed as described.

#### MICROCHEMISTRY

Whenever possible macrochemical methods were checked by microchemical tests. Where several reliable microchemical tests are known for a substance all were employed, although in the discussion hereafter only the principal one is mentioned. In some cases microchemical tests were run, and the amount of substance indicated was so minute that analytical procedure was not deemed profitable. Microchemical methods used were those outlined by ECKERSON (6).

Special attention was given to the Sudan III test for fats and cutin, to the Ruthenium red test for pectic materials, the sulphuric acid-iodine test for cellulose, the solubility tests for cellulose and hemicellulose, and the copper-oxide ammonia test for cellulose (with

special emphasis on the solubility relations of the membrane substances). Various other tests, as outlined by ECKERSON, were also used.

### Results

Since the pods of *Gymnocladus dioica* cling to the tree through the winter, one would expect to find no great difference with increasing maturity in the ease of separation of the pod from the tree, and observation confirmed this supposition. There was a steady increase in the size and weight of the pod and inclosed seeds from July to September. The first signs of change in September were color developments, the light green giving way to a purplish cast. When loss

TABLE I  
ANALYSIS OF THE INNER POD MATERIAL, SAMPLES TAKEN DURING  
LATER STAGES OF MATURITY

| DATE (1927)       | MOISTURE | PERCENTAGE DRY WEIGHT  |                 |                               |   |
|-------------------|----------|------------------------|-----------------|-------------------------------|---|
|                   |          | Ether-soluble fraction | Reducing sugars | Non-reducing sugars (sucrose) | Acid-hydrolyzable (calculated as glucose) |
| August.....       | 75.16    | 3.78                   | 3.3             | 4.2                           | 39.64                                     |
| September 5.....  | 70.11    | 2.19                   | 1.91            | 8.05                          | 59.36                                     |
| September 24..... | 65.79    | 1.87                   | 2.36            | 34.75                         | 9.74                                      |

of water from the pod became greater than water intake, the pod began to turn brown; this usually beginning near the tip of the pod farthest from the attachment to the tree. With this drying of the pod the inner material lost its rather solid cellular structure and became soft and mucilaginous. Accompanying this change the water content of the inner pod material and its total volume decreased (table I). It was noted that at no time during the season did the inner carpellary material occupy all of the area within the pod not taken by the seeds (fig. 1). Experiments to ascertain the possible function of this inner pod material gave interesting results. Green pods were carefully split so that the seeds were uninjured. The inner pod material was removed and the pods (with seeds inclosed) sealed tightly with adhesive tape. After three weeks at room temperature the pods were opened. The seeds had not ripened natu-

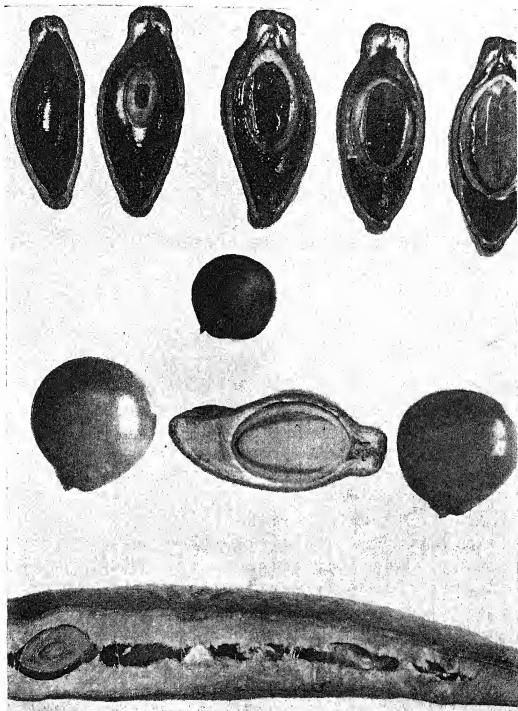


FIG. 1.—Top row, cross-sections through immature pod and inclosed seeds (sections treated with  $I_2$  in KI solution); note abundance of starch, compared with untreated section in center. Large seeds are of average size for immature seeds of maximum water content. Contrast in size between them and mature seed of average size shown just above. Longitudinal section through immature pod shown at bottom of figure. In both cross and longitudinal sections inner pod material does not come in contact with entire circumference of seeds.

rally but had dried unevenly; the Malpighian layer had cracked, and was not a normal dark color.

As shown by table I, the inner carpellary material of the green pod contained considerable starch. The reducing and non-reducing sugar content was then low. At maturity, starch gradually decreased until it was practically absent in the mature pod, and the sugars, especially the non-reducing ones, increased proportionately. Microchemical tests indicated considerable quantities of gumlike materials and saponin, both being present in considerable quantities throughout the later stages of maturity. The latter material is probably largely responsible for the somewhat poisonous nature of the green pod. These tests also checked the results obtained by macrochemical methods recorded in table I. Fig. 1 indicates the amount of starch contained in the green pod.

The immature seeds, with their high water content, were very large. The contrast of these seeds with the mature ones of low water content can be seen in fig. 1, which shows the immature seed at its largest. With the loss of water from the pod and inclosed seeds, the seeds gradually shrank to the size represented by the mature seeds used in this comparison. With this marked shrinking, the formerly pervious seeds became impervious to water, provided the water loss took place gradually and the shrinking was consequently normal and uniform. Removal of the immature seeds from the green pod to a dry atmosphere invariably resulted in quick drying of the Malpighian layer, with consequent cracking.

The degree of maturity of the various pods on the same tree varied if considered at any one period of sampling. There were also variations in the seeds within the same pod.

In any lot of seeds harvested in September, the majority of the seed coats were still soft and pervious to water, although a considerable part of the shrinking had already taken place. These seeds, when placed under favorable conditions, gave practically 100 per cent germination. On the other hand, when these seeds, which contained on the average 15 per cent moisture, were placed under ordinary room conditions they soon reached a water content of 5-8 per cent, and coincidentally gradually became hardened until approximately 95 per cent of them did not imbibe water if placed under conditions

favorable to germination. Tests indicated that the region of the hilum was the last portion to become impervious, and suggested that the shrinking accompanying water loss finally closed the last openings in the region of the old vascular tissue which fed the seed.

Samples of the seed coat were taken in the later stages of maturity and compared with those taken from mature water-impervious seeds. Practically no differences were detected. These results are discussed under the heading of mature seeds.

Marked changes in the oil, soluble carbohydrate, and insoluble carbohydrate content took place in the cotyledons and embryo dur-

TABLE II  
ANALYSIS OF COTYLEDONS (WITH EMBRYO) DURING LATER  
STAGES OF MATURITY

| DATE (1927)      | MOISTURE | PERCENTAGE DRY WEIGHT  |                 |                               |   |
|------------------|----------|------------------------|-----------------|-------------------------------|---|
|                  |          | Ether-soluble fraction | Reducing sugars | Non-reducing sugars (sucrose) | Acid-hydrolyzable (calculated as glucose) |
| July 30.....     | 86.93    | 6.43                   | 1.17            | 27.31                         | 9.54                                      |
| August 27.....   | 74.66    | 7.19                   | 0.00            | 24.57                         | 21.80                                     |
| September 5..... | 71.46    | 16.89                  | 0.00            | 25.82                         | 15.82                                     |

ing the later periods of maturity. As shown by table II, the starch content of the cotyledons, based on total dry weight, rose to a maximum (in August) and then decreased markedly as the oil content increased. Maximum oil content was not reached until the seeds were mature and had dried to a low water content.

The reducing sugar content of the cotyledons was not high at any period in the development of the seed. During the late stages of maturity no reducing sugars were found. The non-reducing sugar content was high throughout the later stages of development. In general, these results are in accord with the work of IVANOW (8) on *Linum* and *Brassica*.

Microchemical tests were run on the seeds during development, and were valuable in providing a check on the macrochemical methods. Since the microchemical tests cannot be considered as quantitative, only one table of results of such tests is given (table III).

## MATURE SEEDS

Preliminary tests on the seeds showed that it was necessary to cut through the Malpighian layer in order to insure a quick intake of water. Seeds uncut, or but superficially cut, remained unchanged in pure water, neither gaining nor losing in weight. One lot of uncut seeds was kept in distilled water for six weeks without changes in weight. During this experiment, in order to prevent errors that might be caused by pollution, the water was changed weekly.

As has been found in many investigations on other impervious seeds, strong acids soon disintegrate the Malpighian layer and offer

TABLE III  
MICROCHEMICAL TESTS OF MATURE SEED

| TEST APPLIED          | COTYLEDONS | MALPIGHIAN LAYER | ENDOSPERM |
|-----------------------|------------|------------------|-----------|
| Fructose.....         | —          | —                | —         |
| Glucose.....          | —          | —                | —         |
| Dextrin.....          | —          | —                | —         |
| Sucrose.....          | ++++       | Trace            | Trace     |
| Starch.....           | +          | —                | —         |
| Fats.....             | +++++      | Trace            | —         |
| Cellulose.....        | +++        | +++++            | —         |
| Araban and xylan....  | ++         | ++               | +++++     |
| Pectic substances.... | +          | +++++            | ++++      |
| Chitin.....           | —          | —                | —         |

an excellent method of preparing the seeds for planting on a large scale. The method consists in placing the seeds in the acid a few minutes (until the outer layer is destroyed) and then washing well in water. This method is used by some Indiana farmers when planting on a large scale.

Using a very hot flame, it was possible to burn the Malpighian layer sufficiently to destroy its water-excluding power without destroying the viability of the seed. It is doubtful whether this method has ever been of the importance that has been attributed to it in the case of the germination of acacia seeds in the vicinity of old campfires. The alkalinity of the ash of such fires would need to be marked in order to exert influence in softening the seed coat. Rapid and violent changes in temperature exerted but slight effect on the water-excluding layer in dry mature seeds. Lots of seeds kept in liquid air



for fifteen minutes and then plunged into warm water remained impervious to water, although the cotyledons were broken into pieces by the treatment. Nevertheless these seeds showed signs of germinating when filed and placed in favorable conditions.

Lots of water-impervious seeds were placed on a medium on which mixed cultures of fungi (including species of *Rhizopus*) were grow-

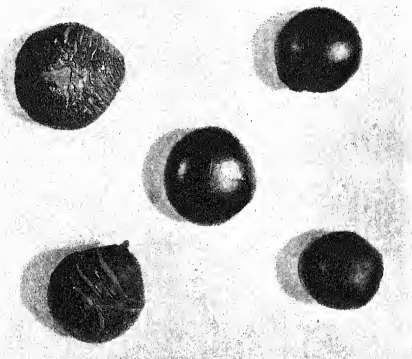


FIG. 2.—Left: seeds showing cracking of Malpighian layer (seeds removed from green, nearly mature pod of high moisture content and dried in atmosphere of low humidity); seed in center is naturally matured, impervious seed; seeds on right are naturally matured, impervious seeds subjected to action of fungous growth; note slight corrosion.

ing, and it was noted that after two weeks some corrosion of the outer coats had occurred (fig. 2). DAVISON and WILLAMAN (5) found that *Rhizopus tritici* produced much protopectinase. To attempt to experiment with enzymes and their action on the seed coat would constitute a special problem in itself, since it would entail the obtaining of rather uncommon enzymes in a somewhat pure form. As stated previously, samples of the Malpighian layer cut from green pervious seeds gave, on analysis, results similar to those obtained

from materials secured from mature seeds filed and soaked in water as hitherto explained. These analyses showed this layer to contain:

|                                       |                  |
|---------------------------------------|------------------|
| Ether-soluble material.....           | 0.53 per cent    |
| Reducing and non-reducing sugars..... | Practically none |
| Insoluble fraction.....               | 96.75 per cent   |

This insoluble fraction, on acid hydrolysis, yielded 20.3 per cent glucose. When samples of the Malpighian layer were analyzed for

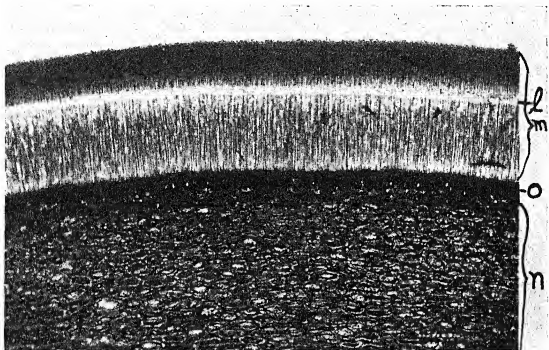


FIG. 3.—Photomicrograph of cross-section through seed coat, showing Malpighian layer (*m*), with light line (*l*), osteosclerid cells (*o*), and portion of nutrient layer (*n*) (section treated with Ruthenium red). Unstained portion of Malpighian layer probably largely cellulose (see fig. 5); portion of coat below Malpighian layer not impervious to water.

pectic materials, as previously explained, the yield calculated as calcium pectate amounted to 22 per cent of the dry weight of the sample.

As shown by table III, microchemical tests substantiated the analytical data. With Sudan III only the outermost portion of the Malpighian layer gave a test. In some seeds a negative test was recorded. The Malpighian layer gave a positive test for hemicellulose, using phloroglucin-HCl, and for cellulose using strong  $\text{H}_2\text{SO}_4$  and  $\text{I}_2$  in KI. It gave a marked test for pectic materials, as indicated by the

Ruthenium red test (figs. 3, 4). The latter figure shows the amount of pectic material found about each cell of the Malpighian layer. Fig. 5 shows the action of strong sulphuric acid and  $I_2$  in KI on a similar section. Note that the area stained by Ruthenium red remains colorless in this case. Impervious seeds boiled in  $N/30$  HCl gave quantities of jelly-like substance. Check lots boiled in distilled

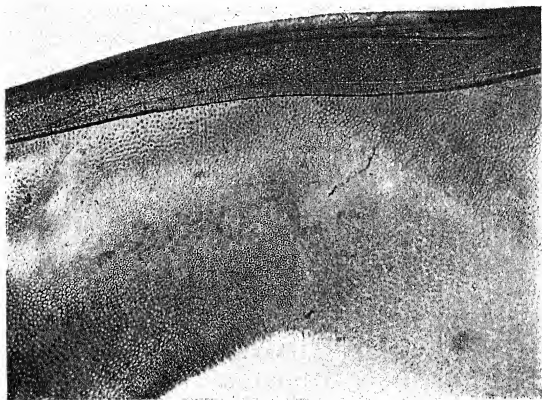


FIG. 4.—Photomicrograph of section through Malpighian layer at right angles to longest diameter of cells (tangential to seed coat surface); section treated with Ruthenium red; darker portion is pectic material, portion not stained is cellulose.

water for a similar period yielded but a small amount. Impervious seeds were kept in fresh copper-oxide ammonia for several days without any noticeable effect on the permeability of the Malpighian layer.

The dry residue of this layer, from which the materials soluble in the HCl and ammonium citrate had been extracted in the quantitative tests for pectic materials, still showed clearly the light line in those particles where a number of cells remained closely packed together, as in the normal untreated tissue (fig. 6). The outer portion

of the Malpighian layer had been dissolved away. Those cells which still clung together could easily be separated by applying slight pressure on the cover glass when examining particles with the microscope. Treatment of this material with any of the cellulose reagents gave a positive test. Fig. 6 shows the response of these cells to strong sulphuric acid and  $I_2$  in KI solution. The slide from which this

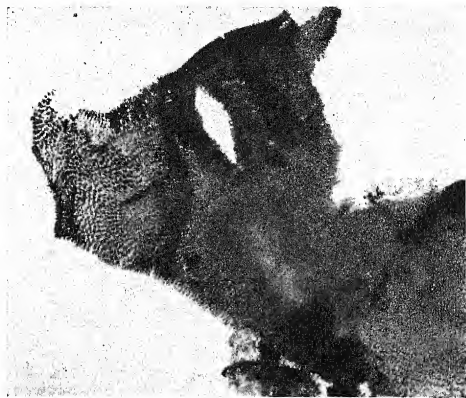


FIG. 5.—Photomicrograph of section similar to one shown in fig. 4, but treated with strong sulphuric acid and  $I_2$  in KI solution, instead of Ruthenium red (note that area colored by Ruthenium red in other section is not colored here); color with sulphuric acid and  $I_2$  in KI solution indicates cellulose. Quick destruction of tissue made good reproduction of this test difficult.

photomicrograph was taken was treated with the acid solution and the action halted at this stage by the application of water. The swelling of the cells below the light line, as contrasted with the slight action in its region, can easily be noticed. This material quickly dissolved in copper-oxide ammonia, using the same strength of reagent which had no effect on the untreated impervious seeds.

An analysis was made on the nutrient layer of the seed coats with the following results:

|  |                |
|--|----------------|
| Ether-soluble fraction.....            | 0.49 per cent  |
| Sugars, reducing and non-reducing..... | Traces         |
| Insoluble material.....                | 96.88 per cent |

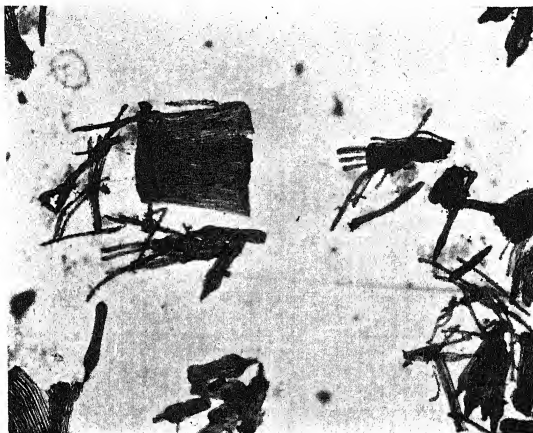


FIG. 6.—Photomicrograph of residue after extracting pectic material from finely ground Malpighian layer (note light line in particle in which several cells still cling together, and, by comparing with fig. 3, that outer portion has been dissolved by pectic solvents). Bottle-shaped objects are individual cells placed on slide after being acted upon for 2 minutes by strong sulphuric acid (with  $I_2$  in KI solution). Note that portion of cell in region of light line and slightly above is more resistant to action of sulphuric acid than remainder of cell.

This insoluble material yielded on acid hydrolysis 13.81 per cent glucose. The nutrient layer played no part in water exclusion, and the analysis indicated that it was not of great importance as a storage place for food material, although it did undoubtedly play a big

part in the maturing seed and the formation of a perfect Malpighian layer.

The endosperm was shown by the analysis to be largely hemicellulose, which yielded on hydrolysis as high as 75 per cent of its dry weight as glucose. The ether-soluble fraction amounted to 0.49 per cent. Sugars were present in traces.

The cotyledons (and embryo) of the mature seed gave the following analysis (percentages based on dry weight):

|  | PER CENT |
|--|----------|
| Moisture.....                                | 5.18     |
| Glucose.....                                 | 0.00     |
| Ether-soluble fraction.....                  | 22.11    |
| Sucrose.....                                 | 13.42    |
| Acid-hydrolyzable fraction (as glucose)..... | 13.34    |

These figures agree in most respects with those of BARKENBUS and ZIMMERMAN (1). The oil extracted had a specific gravity of 0.921, an iodine number of 137.1, and a saponification number of 201.1.

TABLE IV  
ANALYSIS OF COTYLEDONS (WITH EMBRYO) FROM GERMINATING SEEDS

| DAYS UNDER<br>CONDITIONS<br>FAVORABLE FOR<br>GERMINATION | GROWTH IN<br>LENGTH  | MOISTURE | PERCENTAGE DRY WEIGHT         |                    |   |  |
|--|----------------------|----------|-------------------------------|--------------------|---|--|
|  |                      |          | Ether-<br>soluble<br>fraction | Reducing<br>sugars | Non-<br>reducing<br>sugars<br>(sucrose) | Acid-hydro-<br>lyzable<br>(as glucose) |
| 4.....   | Hypocotyl<br>2.5 cm. | 70.81    | 11.23                         | 0.12               | 5.37                                    | 19.6                                   |
| 10.....  | Plumule<br>4.5 cm.   | 67.09    | 10.73                         | 0.144              | 6.57                                    | 34.3                                   |
| 18.....  | Plumule<br>7.5 cm.   | 66.96    | 7.22                          | 0.12               | 8.64                                    | 30.2                                   |

Table IV indicates that the results of analyses of the cotyledons (embryo) of the germinating seed were the reverse of those in the maturing cotyledons. With germination, the oil content of the cotyledons dropped rapidly and starch increased proportionately. Reducing sugars increased but remained low. Non-reducing sugars continued to constitute a considerable part of the food reserve. The seed contained a high sucrose content throughout the later period of maturity and through the stages of germination.

With germination, the iodine number of the oil dropped to 93.94 after six days of growth. The oil content of the cotyledons decreased so rapidly with germination that it was difficult to obtain samples for determination of the iodine number after germination had progressed for some time. These results with the germinating seed are in accord with the findings of LECLERC DU SABLON (10) on oil-containing seeds.

That the endosperm played a considerable part in providing food for the growing embryo was evident from the fact that it had practically disappeared in the seedling which had been growing ten days. At that time the remains of the seed coat still clung around the cotyledons beneath the soil. At that stage the cotyledons were well filled with starch (table IV). Fig. 7 represents graphically the data recorded in tables II and IV, showing the changes which take place in the cotyledons during the later stages of maturity and during germination.

#### Discussion

Since the inner pod material of *Gymnocladus dioica* is seldom in contact with the entire surface of any seed within the pod, it is scarcely possible that it contributes any water-excluding substance to the seed coat. Sections made through the seed coat at different periods of the seed's development showed the same general cellular structure. These lines of evidence indicate that the idea suggested by NELSON (14) for some of the other legumes does not hold for *G. dioica*.

The results obtained in removing the inner carpellary material suggest that this material plays a part in insuring water-impervious seeds by preventing quick drying out of the pod and seed, when the water supply from the tree ceases. It is evident that the gumlike material is well suited to slow uniform water loss. Quick drying of green seeds can lead to but one result, namely, a badly cracked Malpighian coating that cannot be impervious to water.

It is difficult to ascribe the change in the maturing seed, from a condition where water is not excluded to a condition of complete exclusion, to any one particular phenomenon. The fact that no difference could be noted in the analysis of the Malpighian material cut from the green seed as compared with the analysis of material from

the mature impervious seed indicates that the chemical response to the drying of the seed may be minor, and that the physical feature of uniform shrinking may be more important. It is entirely possible,

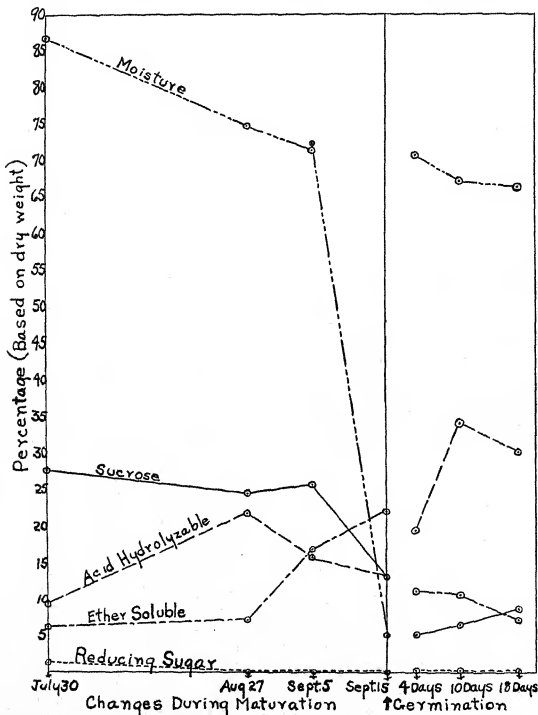


FIG. 7.—Chemical changes in cotyledons (with embryo) during maturity and germination; September 15 chosen as date of maturity although date at which seeds became impermeable varied with method of storing pods.



however, that chemical changes might take place which could have considerable influence on water exclusion, yet have no effect on the materials extractable by the alcohol-ether-water method. Similarly, it is possible that there might be considerable change in the pectic materials with desiccation, yet in both cases they would be easily extracted by the method used. It is not unlikely, judging from present knowledge of many plant materials, that desiccation would tend to force the acids contained in the pectic material toward the anhydride form. Such forms tend to be less pervious to water. That such a change might take place here is difficult to prove.

The large quantitative yield of calcium pectate when the Malpighian layer was analyzed for pectic material, the very pronounced test with Ruthenium red, the solubility test with copper-oxide ammonia, and the ease with which jelly-like material could be split off from impervious seeds in boiling dilute HCl, all suggest that pectic materials are responsible for water exclusion. That these materials must be water-insoluble, at least in the outer portions of the coat, is assumed since if they were soluble some loss would have been noted in the lots of seeds kept for long periods in distilled water.

In the present uncertain state of our knowledge concerning pectic materials, it is best not to draw sweeping conclusions concerning the exact nature of the chemical compounds which make possible water exclusion.

Two lines of evidence indicate that in *Gymnocladus dioica* the light line is not alone responsible for water exclusion. In the first place, filing through the light line does not secure the ready absorption of water by the seed. Also, in remains of the Malpighian material (from which all substances soluble in the solvent used in the analysis for pectic materials had been removed) the light line is plainly visible. All of this material disintegrated after a few minutes in copper-oxide ammonia. If the chemical and physical conditions which produce the light line were alone responsible for water exclusion, it would seem logical to expect that copper-oxide ammonia would soon disintegrate the hard seed coat. The fact that this reagent had no effect on impervious seeds indicates that neither cellulose nor hemicellulose is directly responsible for water exclusion. These statements do not preclude the probability that in many of

the smaller leguminous seeds the materials which are responsible for water exclusion are located in the light line. No work was done on seeds other than *Gymnocladus dioica*.

In this work the term light line is reserved for the lower, narrow, well defined line to be seen in fig. 3, and is used in no case to refer to the upper, broad, less well defined line mentioned as a second light line by PAMMEL in his earlier work (16).

Since in any lot of seeds which have apparently developed normally there will be found a small percentage pervious to water, it is logical to assume that there are varying degrees of perfection in the water-excluding layers. Some will become pervious with but slight alterations in the coat; others may continue to exclude water even after much of the coat has been lost by abrasion or digestion through fungus action.

The changes in the reserve food materials in the seed of *Gymnocladus dioica* are not unlike those which have been found to occur in many oil-containing seeds. It has long been recognized that there is a definite cycle in the building up and later in the utilization of oils as food material in the plant. The data gathered on *G. dioica* substantiate the results of others, in that the steps in utilization of oils during germination consist in a series of changes similar to those occurring in the synthesis of the oils, except that they are in the reverse order. It is interesting to note that the actual synthesis of oils takes place in the later stages of maturity, and primarily at the expense of the starch reserve in the seed. Inversely with germination, the starch content increases rapidly and markedly at the expense of the stored oil. The decrease in the iodine number of the oil with germination indicates that the fats of the unsaturated series are more readily utilized.

The non-reducing sugar content of this seed and of the inner pod material is high. The fact that the invertase method was used in the determination of the non-reducing sugars should preclude the possibility of other substances, such as glucosides, interfering in the final result.

The reducing sugar content of the mature cotyledons is practically zero; the total quantity of such sugars, in both the maturing and the germinating stages, is very low, a fact that indicates that these materials are constantly being utilized.

### Summary

1. In *Gymnocladus dioica* the impervious seed coat is closely associated with the physical phenomenon of uniform shrinking.

2. The inner pod material, which in the nearly mature pod forms a gumlike mass, plays a part in insuring a slow uniform drying of the seed.

3. Tests indicate that from a chemical standpoint insoluble pectic materials may play a big part in water exclusion by the Malpighian layer.

4. In the present uncertain state of knowledge concerning the composition of the pectic materials, it is not possible to tell whether chemical changes in the Malpighian layer accompany shrinking in the final stages of rapid water loss when the seed becomes impervious.

5. Starch is abundant in the cotyledons in the immature stages and during germination.

6. In the cotyledons the maximum oil content is reached at maturity; at that time the starch content reaches a minimum.

7. Non-reducing sugar is an important storage form in the seed.

8. Reducing sugars are absent in the mature seed, but are found in small quantities in the immature and the germinating stages.

The writer wishes to express his appreciation of the advice and valuable suggestions of Professor C. A. SHULL and Dr. S. V. EATON, and for the encouragement and inspiration gained from contacts with members of the Botany Department of the University of Chicago.

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### LITERATURE CITED

1. BARKENBUS, C., and ZIMMERMAN, A. J., Kentucky coffee nut tree oil. Jour. Amer. Chem. Soc. 49:2061-64. 1927.
2. BERGTHEIL, C., and DAY, D. L., On the cause of hardness in the seeds of *Indigofera arrecta*. Ann. Botany 21:57-60. 1907.
3. COE, H. S., and MARTIN, J. N., Structure and chemical nature of the seed coat and its relation to impermeable seeds of sweet clover. U.S. Dept. Agric. Bull. 844. Pt. II. 1920.

4. CONRAD, C. M., A biochemical study of the insoluble pectic substance in vegetables. Amer. Jour. Bot. 13:531-547. 1926.
5. DAVISON, F. R., and WILLAMAN, J. J., Biochemistry of plant diseases. IX. Pectic enzymes. BOT. GAZ. 83:329-361. 1927.
6. ECKERSON, S. H., Outline of microchemistry (unpublished).
7. EWART, A. J., On the longevity of seeds. Reprinted from Proc. Roy. Soc. Victoria 21:(n.s.) Pt. I. 1908.
8. IVANOW, SERGIUS, Über den Stoffwechsel beim Reifen ölhaltiger Samen mit besonderer Berücksichtigung der Ölbildungsprozesse. Beihefte Bot. Centralbl. Abt. I. 28:159-191. 1912.
9. KOCH, F., Outline for tissue analysis (unpublished).
10. LECLERC DU SABLON, M., Recherches sur la germination des graines oléagineuses. Revue Gén. Bot. 7:258-69. 1895.
11. MARTIN, JAMES H., *Gymnocladus canadensis*. Amer. Jour. Phar. 64:557-559. 1892.
12. MARTIN, J. N., The structure and development of the seed coat and causes of delayed germination in *Melilotus alba*. Iowa Acad. Sci. Proc. 29:345-346. 1922.
13. MELL, S. S., *Gymnocladus canadensis* Lamarck. Amer. Jour. Phar. 59:230. 1887.
14. NELSON, A., Hard seeds in Leguminosae. Nature 4:804. 1926.
15. OHGA, I., The germination of century-old and recently harvested Indian lotus fruits, with special reference to the effect of oxygen supply. Amer. Jour. Bot. 13:754-759. 1926.
16. PAMMEL, L. H., On the structure of the testa of several leguminous seeds. Bull. Torr. Bot. Club 13:17-24. 1886.
17. ———, Anatomical characters of the seeds of Leguminosae, chiefly genera of Gray's manual. Trans. Acad. Sci. St. Louis 9:91-274. 1899.

# CYTOLOGICAL STUDY OF THE ZOOSPORES OF BLASTOCLADIA<sup>1</sup>

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(WITH TEN FIGURES)

## Introduction

Very little work has been reported concerning the details of structure of the zoospores of the fungi. The morphology and cytology of the zoospores occurring in a number of genera of algae have attracted much more interest, and have been reported in the literature in some detail. DEBARY (3) described the cilia as springing from a definite spot in the surface of the zoospore as processes of the peripheral layer of protoplasm. ROTHERT (22) showed that the cilia in *Saprolegnia* appear at first as slow outgrowths like little short, straight bristles which show faint oscillations. STRASBURGER (23, 24) described the structure of the zoospores of *Oedogonium*, and showed a small refractive granule at the base of each cilium. He also discussed the cilia-bearing organs of several other genera, including *Cladophora* and *Vaucheria*, in which connection he brought out the idea of a swelling of the plasma membrane at the point of insertion of the cilia. TIMBERLAKE (26), working with *Hydrodictyon*, reported that the cilia grow out from a small body lying near the plasma membrane, and that this body is connected with the nucleus by a delicate protoplasmic strand. DANGEARD (8) studied the zoospores of *Polytoma* and showed the cilia to be inserted in a nodule which is in contact with the surface of the spore, and that a chromatic thread connects this nodule with the nucleus. DAVIS (10) showed that in the zoospores of *Derbesia* the nucleus migrates toward the plasma membrane, and granules are found in radiating cytoplasmic strands apparently moving outward toward the plasma membrane, where by fusion of the numerous granules they form a ringlike structure from which the cilia grow. GRIGGS (13) studied the zoospores of *Rhodochytrium* and found a

<sup>1</sup> Paper no. 311, from the Department of Botany of the University of Michigan.

deeply stained body at the base of the cilia which is connected with the nucleus. BARRETT (2), working with the plant now recognized as *Allomyces arbuscula*, showed the cilia attached to the nucleus but did not show a refractive granule at the insertion of the cilia. He showed that the spores may at times be binucleate but did not show the cilia in these binucleate spores. ENTZ (11) investigated the same species of *Polytoma* studied by DANGEARD, and reported that the two flagella are inserted in two separate blepharoplasts, which are connected with each other by a strand (the lateronema), and with the nucleus by another strand (the rhizonema). HARTMAN (14) reported that previous to the formation of cilia in *Eudorina*, the pointed end of the nucleus (centriole) lies close to the anterior pole of the cell, and then draws back to the interior of the cell, leaving behind a double basal body from which the cilia grow. COUCH (7) states:

the cilia in *Leptolegnia* are formed by the thinning down of the parietal membrane between the spore origins into one or two strands which connect the spores, as a rule, until after discharge. Upon discharge these threads are broken and as the spore reshapes itself they move around to occupy the normal position of cilia.

NISHIMURA (19) observed that in the zoospores of *Plasmopara halstedii* the cilia sprout from the beaklike apex of the nucleus at the spot where a well stained granule can be distinguished. This granule is believed to be the blepharoplast.

The zoospores of *Blastocladia* have been described at various times as having cilia varying from one to three in number. THAXTER (25) gave a rather detailed description of *B. pringsheimii*, which had been partially described by REINSCH (21) in 1876. THAXTER described the zoospores as being typically biciliate, stating, however, that "in some instances it has been found impossible to make out more than a single cilium even after the zoospore was stained." PETERSEN (20) described the zoospores of this plant as being uniciliate. MINDEN (18) considers the uniciliate spores to be the typical ones, but states that he has observed spores with two and rarely with three cilia. KANOUSE (16) described the zoospores of *B. globosa* Kanouse as being biciliate.

In view of these discrepancies in the description of the zoospores

of the same genus, and even of the same species by different investigators, it is evident that further study of the details of the structure of these spores is necessary. The present paper gives a physiological explanation of this variation in the morphology of the zoospores of *B. pringsheimii* and *B. globosa*. The cytology of these uniciliate, biciliate, and triciliate spores will be discussed with reference to their structure and the relation of the nuclei to cilia formation.

The plants used in this study were provided and identified by Dr. BESSIE B. KANOUSE. The work, excepting the perfection of the staining technique, was carried on in the cryptogamic laboratories of the University of Michigan under the direction of Dr. C. H. KAUFFMAN, to whom the writer wishes to express his sincere appreciation for many helpful suggestions and for the use of the facilities of the laboratory.

#### Material and method

Collections of the plants were made from a pool in a greenhouse of the botanical gardens of the University of Michigan. This pool was of course accessible during all seasons. Collections were made by exposing fruit bait (apples), using the method described by KANOUSE (15). The bait was exposed from 14 to 21 days, depending on whether young or older material was desired. The shorter exposures were found to be more satisfactory on account of the increased accumulation of other organisms around the tufts of *Blastocladia* with increased exposure. The temperature of the pool varied from 12° to 14° C. The material was brought to the laboratory and kept at 10°-12° C. until used, which in all cases was within a few hours after collection. In preparation for obtaining zoospores, the plants were thoroughly washed for 1 hour through four changes of sterile, well aerated conductivity water. The material was then placed in hanging drop mounts and watched under the microscope until numerous zoospores were liberated.

#### KILLING

The zoospores were killed during the height of their activity in hanging drops on no. 1 cover glasses, by exposing the hanging drop containing the zoospores to the fumes of 1 per cent osmic acid. The time of exposure varied from 15 seconds to 2 minutes. The longer

exposure to the osmic acid fumes darkens the material which for some parts of the study is desirable. The shorter exposures, 15 seconds to 1 minute, produce the most satisfactory results for most of the detailed studies.

#### STAINING

The staining technique used in this study was perfected by the writer in connection with some unpublished work carried on at Montana State College.

A number of stains were used, including orange G, safranin, gentian violet, and crystal violet. The most satisfactory and uniform staining was accomplished by the use of the National Aniline Chemical Company Standardized, crystal violet in 0.005 per cent aqueous solution. The solution was prepared for use by adding 0.1 cc. of a 1 per cent stock aqueous solution of crystal violet to 20 cc. of distilled water. After the hanging drop containing the highly active zoospores was exposed to the fumes of osmic acid, an equal volume of dilute staining solution was added to the zoospore suspension. The preparation was allowed to evaporate to dryness at 22°-24° C. This was accomplished satisfactorily on the laboratory table in 18-24 hours when the relative humidity was somewhat low, such as was found to be the case at Bozeman, Montana. In more humid climates, however, as was the case at Ann Arbor, in order to obtain satisfactory results it was necessary to accomplish at least the last part of the evaporation in a desiccator. It was found that satisfactory results were obtained after the preparation had remained in a desiccator over sulphuric acid for 24-36 hours. After its removal the stained area was extracted and cleared in clove oil. When the desiccator was opened, all preparations to be mounted for study at this time were removed and clove oil dropped on the stained area with the least possible delay, to prevent reabsorption of moisture from the air. The extraction in clove oil may be most satisfactorily controlled under the microscope. After a few preparations with a new stain have been cleared and mounted, a satisfactory time control can be worked out. In general, extraction in several changes of clove oil for a few minutes beyond the time when stain extraction is no longer apparent to the naked eye will produce satisfactory results. Changes of clove oil were made by draining off the oil on



the cover glass and gently flooding with more oil. After extraction and clearing, the oil was removed with xylol from the preparation which was mounted in balsam in the usual manner. All detailed studies and drawings were made with the aid of a 2 mm. apochromatic oil immersion objective, a 12 $\times$  compensating ocular, and a camera lucida.

#### Temperature in relation to zoospore formation

To obtain normal and perfect development of an organism or some phase of its life history, it is important and often necessary to provide certain definite favorable environmental conditions. This is true in the study of the normal development and typical structure of the zoospores of the Oomycetes, and especially true among the more highly sensitive genera and species of the group.

The question naturally arises as to what may be interpreted as the normal development and typical structure in a plant when many of the details of its life history are as yet unknown. Normal development may be defined as the development which takes place under optimum conditions. Optimum conditions for spore production are those which enable the greatest possible number of active spores to be formed and liberated in the briefest given time. The spores which develop under optimum conditions are interpreted as exhibiting the morphology and behavior typical for the species.

The plants *B. pringsheimii* and *B. globosa* are strikingly similar in the relation of temperature to the formation of zoospores. In *B. globosa* the minimum temperature is found to be between 5° and 7° C., the optimum between 11° and 13° C., with rapid decrease in the rate of development at 16° C., and slower decrease until the maximum temperature is reached at 21°–23° C. In the plants of *B. pringsheimii* studied, the minimum temperature seems to be the same as for *B. globosa*, with a slightly higher optimum of 12°–14° C., and a maximum temperature which lies between 22° and 24° C. At the optimum temperature large numbers of spores are liberated between 1 and 2 hours after washing is completed, while at temperatures other than the optimum, the time elapsing between completion of the washing process and appearance of the first spores is increased and the continued rate of liberation is diminished.

These variations in temperature relations between the two species are no more than one would expect to find between varieties of the same species, so that for all practical purposes they may be regarded as physiologically similar. In studying the zoospore formation in these plants at various temperatures the most remarkable differences are noted. More than double the number of spores are formed in a given time at the optimum temperature than at a temperature but two degrees removed. There is also a distinct difference in the activity of the spores at the different temperatures. In the lower ranges the movement becomes very sluggish, while as the temperature is increased above the optimum an increased excitement and a less regular movement are noted. The motion becomes more jerky and excited and soon ceases. At the optimum temperature the motion is quite even and uniform, and the length of motility at this temperature seems to be almost unlimited. With sudden variation of the temperature from the optimum, many of the spores may settle and come to rest. If the temperature is again brought to the optimum, many of the spores which have come to rest are seen to become active again and to begin swimming.

### Morphology and cytology

For this, the main part of the study, motile spores were killed and stained by the method described. Material was killed, mounted, and studied from each of the temperature relations already discussed. The following discussion is based on spores formed and liberated at optimum temperatures and at temperatures above the optimum. There appears to be little if any variation from the normal in spores formed below the optimum, except in activity; therefore no special reference will be made to these low temperature spores.

The spores of the collections of *B. pringsheimii* made at Ann Arbor exhibit in general the characteristic shape and size described by THAXTER (25). They are, however, typically uniciliate when allowed to develop normally under optimum conditions. THAXTER has described the zoospores of this plant as follows: "In general form they are oval or elliptical and are, at least in many cases, biciliate: the two cilia arising side by side from the smaller end of the spore." He further states that "in some instances it has

been found impossible to make out more than a single cilium even after the zoospore was stained"; but says "the presence of two has been determined definitely in so many cases that this number may be considered as typical." The nucleus is described by THAXTER as "very large and subtriangular in outline, its base connected with that of the cilia by a fine strand of granular protoplasm. . . . In front of the nucleus lies a broad and distinct mass of granular protoplasm while small groups of granules occur here and there around in the otherwise nearly clear cytoplasm." The size of the spores from the plants collected in 1896 is given as about  $7 \times 5 \mu$ . The measurements of spores from the Ann Arbor collections are  $6-8 \times 5.5-6.5 \mu$ .

The large subtriangular nucleus in the more deeply stained spores shows a dark, denser portion near the broader or distal end opposite the insertion of the cilium, which is separated from the denser chromatic body at the tip by a nearly clear zone (fig. 1). This distal denser portion of the nucleus in less deeply stained material resolves itself into irregular masses of chromatin variously distributed in the peripheral part of the nucleus just inside the nuclear membrane (figs. 3, 4). The forces achieving this wide dispersal of the peripheral chromatin cause the enlargement of the nucleus and hold tense this part of the nuclear membrane. The denser chromatic body has evidently migrated toward the periphery of the spore where the cilium is inserted on the plasma membrane. This movement of the denser chromatic body away from the peripheral chromatin of the nucleus toward the position of the blepharoplast probably results in a stretching of the nuclear membrane in that direction, bringing about the subtriangular shape of this organ.

The cilium is inserted on the plasma membrane in a highly refractive body, the blepharoplast, which is definitely connected to the tip of the nucleus by a single threadlike connection (fig. 2). Occasional spores show two separate threadlike connections between the blepharoplast and the nucleus (fig. 6). The cilium of the spores of this and other species studied is unusually long, being usually from three to four times the length of the long axis of the spore, measuring more than  $30 \mu$  in length.

Between the broad end of the nucleus and the surface of the spore a rather well defined, dense, very finely granular mass of cytoplasm may be distinguished. This dense mass of cytoplasm in some of the

more deeply stained material is so clearly differentiated that it appears almost like a cap covering the end of the spore opposite the insertion of the cilium. At times it is drawn out into a rather acute tip. Fig. 6 shows a zoospore of *B. globosa* with such a caplike cytoplasmic differentiation. This is evidently the structure referred to by THAXTER as the broad and distinct mass of granular protoplasm lying in front of the nucleus.

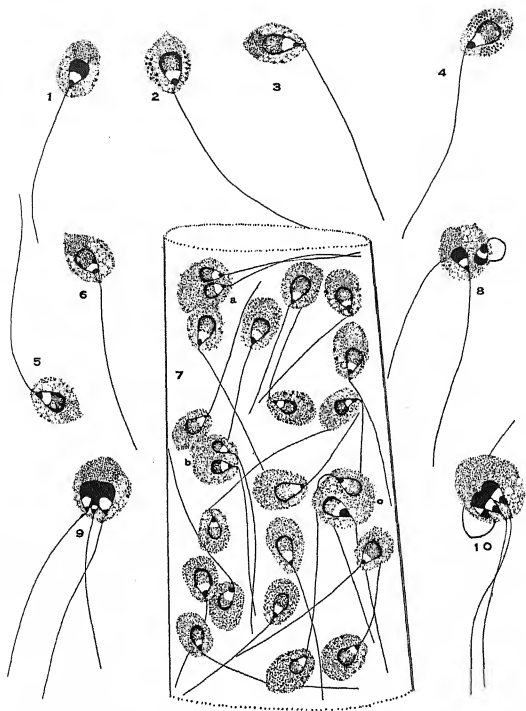
The cytoplasm which is found at the end of the spore surrounding the point of insertion of the cilium and the tip of the nucleus is much less dense than that at the opposite end, and is filled with many small vacuoles. An occasional deeply stained granule is found in this loose network of cytoplasm.

There is a broad equatorial band of dense cytoplasm separating the very dense distal portion from the highly vacuolated cytoplasm surrounding the beaked end of the nucleus and the insertion of the cilium. This equatorial band surrounds the nucleus but is separated from it by a zone of more highly vacuolated cytoplasm. The dense part of this band, in favorable material, is often completely filled with deeply stained, rather large, nearly spherical granules (fig. 4). In some spores these granules apparently are not distributed uniformly throughout this equatorial band but tend to be grouped in two or three parietal platelike groups (fig. 2). These granules were mentioned by THAXTER in his description of *B. pringsheimii*, and probably correspond to the cell inclusions described by DAN-GEARD and TSANG (9) for the zoospores of several genera of the Peronosporales.

The zoospores of *B. globosa* are quite similar in size and structure to those of *B. pringsheimii*, except that they are slightly larger in the long dimension ( $7.9 \times 5.6 \mu$ ), and their cilia tend to be slightly more delicate than those of the spores of *B. pringsheimii* (fig. 5).

When sporangia of either *B. pringsheimii* or *B. globosa* were allowed to develop at temperatures above the optimum but within their range of possible spore formation, it was found that an increasing number of giant spores<sup>2</sup> were formed. The range of temperature

<sup>2</sup> In a paper which came to hand after the present paper was in press, HANS KNIEP (Bot. Ges. 47:199-212. 1929) has shown in his study of *Allomyces javanicus* n.sp. that similar giant spores occur in his species of *Allomyces* as are here reported for *B. globosa* and *B. pringsheimii*.



FIGS. 1-10.\*—Figs. 1-4, *B. pringsheimii*: figs. 1-3, zoospores with varying density of stain, showing different degrees of detail of nuclear structure; density of cytoplasm of distal ends and highly vacuolated netlike structure of ciliated ends of well differentiated spores; fig. 4, zoospore with peripheral part of equatorial region of cytoplasm with highly stained granules; nucleus nearly clear, showing chromatin around periphery and denser chromatic body in tip, connected by strand to blepharoplast at insertion of cilium. Figs. 5-10, *B. globosa*: fig. 5, zoospore showing similar details to *B. pringsheimii* (fig. 2); fig. 6, zoospore showing caplike structure of dense cytoplasm of distal end of spore and two strands connecting tip of nucleus and blepharoplast; fig. 7, portion of sporangium just before liberation of zoospores; a, b, c, binucleated giant zoospores; fig. 8, binucleated giant zoospore with nuclei pointing almost oppositely; figs. 9, 10, trinucleate, triciliate giant zoospores somewhat over-stained.

\* All figures were drawn with the aid of a Zeiss 2 mm. apochromatic objective, compensating oculars 12 and 15X, and an Abbe camera lucida.

at which these abnormal spores were formed varied from 16° to 22° C. When the sporangia were subjected to these less favorable temperatures complete cleavage of the protoplasm did not take place, resulting in numerous compound spores with two and three nuclei and with the accompanying biciliate and triciliate condition. Fig. 7 shows a portion of a sporangium of *B. globosa*. The protoplasm of this sporangium has recently been divided into zoospores. The maturation of this sporangium took place at 17°-19° C. It will be noted that three giant spores are found in this portion of the sporangium (fig. 7a, b, c), the protoplasm having failed to be completely divided into its normal zoospore units at this temperature, which is 4°-6° above the optimum temperature for normal zoospore formation in this species. Figs. 8, 9, and 10 show giant zoospores of *B. globosa* which were formed in sporangia allowed to mature at slightly above 20° C. It will be noted that at this temperature the binucleated and trinucleated spores are formed, with the accompanying increased number of cilia. This temperature (20°-21°) probably approaches rather closely the temperature at which many of the previous investigators studied these species, hence the explanation of the descriptions in the literature of biciliate and triciliate spores occurring in these species. In more densely stained material which had not been thoroughly cleared, the additional nuclei would not be differentiated. Fig. 9 shows a trinucleated spore in which the nuclei were stained too deeply and hence were not well differentiated.

The zoospores in the young developing sporangium of these plants evidently do not have any one definite orientation with relation to each other. It is evident that the nuclei of the spores of fig. 7a, b, and figs. 9 and 10 developed with nuclei oriented in the same manner, and if cleavage had been complete the adjacent spores formed from these giant ones would have pointed in a similar direction to that of their neighbors. Figs. 7c and 8, however, show that the neighboring nuclei contained in these giant spores are pointed in almost opposite directions.

### Discussion

The number and position of the cilia of the zoospores occurring in the lower groups of fungi have been of considerable theoretical

interest, in connection with some of the discussions of the relationships and phylogenetic connections of these fungi. BUTLER (4), discussing characters which are likely to be of phylogenetic significance, states that "another character of importance is the shape of the zoospore and the number and position of its cilia." ATKINSON (1) held that since it is known that there is great variability in the number and position of cilia in the zoospores of the species of certain genera, the recognizing of phylogenetic series among the lower fungi based on the number of cilia possessed by the zoospores does not appear to be sound. MINDEN (17) makes use of the number and position of the cilia in his characterization of the families of the Saprolegniales. KANOUSE (16), in characterizing the orders Blastocladales and Leptomitales, says in her discussion of the former "zoospores usually uniciliate," while in the latter she states that the zoospores are "kidney shaped, mostly biciliate." GÄUMAN and DODGE (12), in discussing Blastocladiaceae, state that "this family is reminiscent of the Leptomitaceae from which it differs by its typical uniflagellate zoospores." COOK (6), agreeing with ATKINSON, says "it seems reasonable therefore to maintain that flagellation is not a fundamental character of classification."

It seems evident, from the studies of the zoospores of the two species of *Blastocladia* reported in this paper, that the cilia are constant in number and position when allowed to develop normally under conditions most favorable for zoospore formation. This evidence very definitely supports BUTLER's views as already quoted; and the usage of MINDEN, KANOUSE, and GÄUMAN and DODGE of zoospore characters, including number of cilia, in considering phylogenetic relationships or differences of these lower fungi.

The designation of the large subtriangular body in the zoospores of *Blastocladia* as a nucleus following THAXTER's original description will be noted, which in view of the evidence at hand the writer believes to be correct. BARRETT (2), in his description of the rather closely related plant now recognized as *Allomyces arbuscula* Butler, designates this nucleus as the central body, and as the reserve food body. It would appear that BARRETT's figs. 41 and 42, showing zoospores killed in iodine and Flemming's solution respectively, show nuclei of different size, or probably a single and a binucleated spore,

rather than structures of different nature. His fig. 42, showing the larger nucleus, also exhibits two cilia, which would indicate the presence of two nuclei which were not differentiated by the methods used in the preparation from which the figure was made. The large nucleus of the zoospores of these plants is very evidently inclosed by a membrane, or it would not be pulled out to a point by the seeming attempt of the nucleolus to move toward the plasma membrane at the insertion of the cilium. It is evident that the nuclei of the spores of these plants correspond very closely, except in size and number of cilia attached, to the beak-shaped nuclei of the young zoospores of *Plasmopara halstedii* (Farl.) Berl. & de Toni described by NISHIMURA (19). The developing spores illustrated by BARRETT (2) exhibit nuclei in several cases suggesting the beaked nuclei of the spores of *Plasmopara* figured by NISHIMURA, which were nearing the same stage of maturity.

The important relation of the nuclei of these spores to cilia formation becomes more evident when it is realized that in the study of hundreds of uninucleate spores none were found bearing two cilia, and in all cases where spores were found to contain more than one nucleus the accompanying increased number of cilia was always found. The uniciliate spores are undoubtedly the typical ones for these species, since at temperatures most favorable to zoospore formation it was almost impossible to find any spores having more than one nucleus.

TIMBERLAKE (26), in discussing the formation of giant spores in *Hydrodictyon*, states: "but in some cases the separation may take place before cleavage is complete with the result that large binucleate masses form spores directly." He further states that "in the giant swarm spores there is a complete locomotor apparatus consisting of cilia, basal body and connecting threads connected with the nucleus." TIMBERLAKE's fig. 39 shows these giant spores. It would be interesting to know more about the temperature in relation to the formation of normal and giant spores in this genus. It is entirely probable that a relation may exist similar to that reported in this paper for *Blastocladia*, since in this study of *Hydrodictyon* the plants were removed from their natural habitat to battery jars in the laboratory, which may have brought about a temperature in the cultures unfavorably high for normal zoospore formation.



The data given by the various investigators who have studied collections of *B. globosa* and *B. pringsheimii* make the fact evident that some of the studies and measurements of zoospores were based on normal, typical material, while other studies and measurements were evidently also based on observations of giant spores. This has led to misunderstandings and at times misinterpretation of life histories, and of the proper position of these fungi with reference to other related groups. The discrepancies pointed out in the first part of this paper are therefore the results of working with material kept under unfavorable conditions for the development of normal and perfect zoospores typical of the species.

PETERSEN (20), in his discussion of *B. pringsheimii*, states that "according to the experiences of THAXTER and myself, it is evident that the zoospores but seldom escape from the sporangia. THAXTER ascribes this circumstance to the numerous bacteria which rapidly settle around the individuals of this species."

Since there is no mention by either THAXTER or PETERSEN of the temperatures under which these observations were made, we may be safe in assuming that their studies were conducted at ordinary room temperatures, probably varying around 21° C. or even higher, which is well toward the upper limit for zoospore formation in this plant. The inability of the zoospores to escape from the sporangia of the plants studied by THAXTER and by PETERSEN was evidently due to temperatures unfavorably high for zoospore formation, at which the plants under observation were kept, rather than to the presence of numerous bacteria. One might think upon superficial examination that possibly the reason for the low optimum temperatures for the formation of zoospores in these plants could be explained by the fact that these temperatures inhibit the rapid development of the bacteria which collect around the plants of *Blastocladia*, thus giving the plants more freedom to develop normally. This cannot be true, since as we subject the plants and zoospores to temperatures but slightly lower than the optimum for zoospore formation and activity, the activity of the zoospores and the ability of the plants to form zoospores rapidly diminish, while there is of course less bacterial activity at these temperatures than at or above the optimum temperatures for the formation of zoospores. Furthermore, BUTLER (5), in his studies of the rather closely related plant

*Allomyces arbuscula*, states that "it grows freely in such cultures and is not much hindered by the development of bacteria as in some of the allied forms."

The importance of complete control of environmental factors, when studying the delicate plants of these highly sensitive groups, becomes more evident as one understands more of their life history. Further studies of the details of the morphology and cytology of the zoospores of other genera of the lower fungi, allowed to develop under controlled physiological conditions and stained so as to differentiate their structures, should reveal many facts which have remained unexplained during the decades of study devoted to the fungi.

### Summary

1. The uniciliate condition of the zoospores is typical for the species of *Blastocladia* studied.
2. The cilium is attached to the tip of the nucleus, and there is also a definite blepharoplast at the insertion of the cilium on the plasma membrane.
3. Biciliate and triciliate conditions of zoospores of these species are brought about by incomplete cleavage in the sporangium.
4. Incomplete cleavage of protoplasm in the sporangium is due to environmental conditions, especially temperature, unfavorable to normal zoospore formation.
5. The optimum temperature for zoospore formation in *Blastocladia pringsheimii* and *B. globosa* lies between 11° and 14° C.

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### LITERATURE CITED

1. ATKINSON, G. F., Some problems in the evolution of the lower fungi. *Ann. Mycol.* 7:441-472. 1909.
2. BARRETT, J. T., The development of *Blastocladia strangulata* n. sp. *BOT. GAZ.* 54:353-371. 1912.
3. DEBARY, A., Comparative morphology and biology of the fungi, mycetozoa and bacteria. *Eng. Trans. Oxford.* 1887.
4. BUTLER, E. J., An account of the genus *Pythium* and some Chytridiaceae. *Mem. Dept. Agric. India Bot. Sec. 1.* 5:1-160. 1907.
5. ———, On *Allomyces*, a new aquatic fungus. *Ann. Botany* 25:1023-1034. 1911.

6. COOK, W. R. E., The inter-relationships of the Archimycetes. *New Phytol.* 27:230-260; 298-320. 1928.
7. COUCH, J. N., Some observations on spore formation and discharge in *Leptolegnia*, *Achlya* and *Aphanomyces*. *Jour. Elisha Mitchell Sci. Soc.* 40:27-42. 1924.
8. DANGEARD, P. A., Etude sur la structure de la cellule et les fonctions le *Polytonia uella*. *Le Botaniste* 8:5-58. 1902.
9. DANGEARD, P. A., and TSANG, K. C., Recherches sur les formations cellulaires contenues dans le cytoplasme des Péronosporées. *Le Botaniste* 17 (5 and 6):365-370. 1926.
10. DAVIS, B. M., Spore formation in *Derbesia*. *Ann. Botany* 22:1-18. 1908.
11. ENTZ, G., Über die mitotische Teilung von *Polytoma uella*. *Arch. Protist.* 38:324-354. 1918.
12. GÄUMAN, E. A., and DODGE, C. W., Comparative morphology of fungi (p. 58.) 1928.
13. GRIGGS, R. F., The development and cytology of *Rhodochytrium*. *Bor. Gaz.* 53:128-172. 1912.
14. HARTMAN, M., Untersuchung über die Morphologie und Physiologie des Formwechsels der *Phylomonaden* (Volvocales). *Archiv. Protist.* 43:223-286. 1921.
15. KANOUSE, BESSIE B., The distribution of the water molds, with notes on the occurrence in Michigan of members of the Leptomitaceae and Blastocladiaceae. *Mich. Acad. Sci. Arts and Letters* 5:105-114. 1925.
16. ———, A monographic study of special groups of the water molds. *Amer. Jour. Bot.* 14:287-306; 335-357. 1927.
17. VON MINDEN, M., Chytridiineae, Ancylistineae, Monoblepharidineae, Saprolegniaceae. *Kryptogamenfl. Mark Brandenburg* 5:209-612. 1915.
18. ———, Beiträge zur Biologie und Systematik einheimischer submerseer Phycomyceten. *Mykol. Untersuch. Ber.* 2:146-254. 1916.
19. NISHIMURA, MAKATA, Studies in *Plasmopara halstedii*. *Jour. Coll. Agric. Hokkaido, Imp. Univ.* 17:1-61. 1926.
20. PETERSEN, H. E., An account of Danish freshwater Phycomycetes with biological and systematic remarks. *Ann. Mycol.* 8:414-560. 1910.
21. REINSCH, P. F., Beobachtungen über einige neue Saprolegniaceae, etc. *Jahrb. Wiss. Bot.* 11:283-311. 1887.
22. ROTHERT, W., Die Entwicklung der Sporangien bei den *Saprolegniaceen* Cohns. *Beiträge Biol. Pflanzen.* 5:291-347. 1892.
23. STRASBURGER, E., Schwärmsporen, Gameten, pflanzliche Spermatozoiden und das Wesen der Befruchtung. *Histol. Beit.* 4:47-156. 1892.
24. ———, Reduktionstheilung, Spindelbildung, Centrosomen und Cilienbildung im Pflanzenreich. *Histol. Beit.* 6:1900.
25. THAXTER, R., New or peculiar aquatic fungi. III. *Blastocladia*. *Bor. Gaz.* 21:45-52. 1896.
26. TIMBERLAKE, H. G., Development and structure of the swarm spores of *Hydrodictyon*. *Trans. Wis. Acad. Sci.* 13:486-522. 1902.

## BRIEFER ARTICLES

### OCCURRENCE OF MULTIPLE-SEEDED XANTHIUM IN AUSTRALIA

(WITH TWO FIGURES)

In several previous papers attention has been called to the rare occurrence, under natural conditions, of multiple-seeded or multiple-celled specimens of *Xanthium*. The four instances of the production of these anomalous burs so far reported from the United States have all been from the central valley and eastern great plains region. The first discovery of such burs was at Onaga, Kansas, in 1909; and the subsequent specimens were collected at Richland, Indiana, in 1922; Kansas City, Missouri (70th and Indiana), in 1925; and near Allen, Nebraska, in 1927.

Now, through the kindness of Dr. JEAN WHITE-HANEY and Dr. B. T. DICKSON, four specimens of burs of this same type have been received which were collected May, 1929, at the Wolfgang Station in the Clermont district of Central Queensland, Australia. It seems probable, therefore, that the sporadic occurrence of these unique plants may be expected in any part of the world where cockleburs are found, or at least in localities where two or more species occupy the same region.

Fig. 1 shows the four burs from the multiple-seeded specimen, and also the normal burs of the common pest species of *Xanthium* which grows there. In his letter, Dr. DICKSON refers to this common native species as "*Xanthium chinense* (*pungens*, etc.)." Dr. WHITE-HANEY also seems to think that this particular species is the same as our American form. While she had not had opportunity to trace it systematically, she states that it looks like *X. canadense*, and this in the monograph of MILLSPAUGH and SHERFF is considered a synonym of *X. chinense*. It is believed that the American multiple-seeded forms have all been related genetically to *X. chinense*, which is called by WIDDER *X. pungens*. It is most likely, therefore, that the Australian forms are identical with the American ones. The native bur is known in Australia as the Noogoora bur, two of these normal Noogoora burs being shown in fig. 1. A cross-section of the largest bur in fig. 1, and the seed removed from it are shown in fig. 2.

The value of these multiple-seeded burs as a source of material for cytological and anatomical investigations, and for comparative studies of

normal and abnormal floral development is obvious. Evidences of hybridization should be sought, and chromosome studies made to determine whether the peculiar fruiting behavior is definitely correlated with some

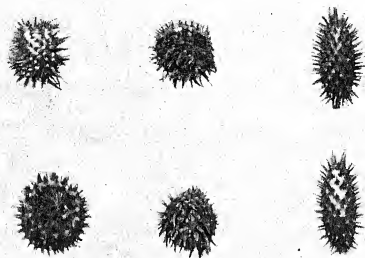


FIG. 1.—Burs of multiple-seeded *Xanthium* from Australia: specimens of Noogoora bur at right, from which multiple-seeded form probably arose.



FIG. 2.—Cross-section of largest multiple-seeded bur of fig. 1, showing circle of ovarial cavities within walls of involucre; only one cavity, the largest, contained a seed, which is shown at right.

specific change in the mechanism of inheritance. If these multiple-seeded forms represent  $F_1$  crosses, as seems possible from the work of Miss SYMONS, some reason should be found for the complete failure of segregation of bur form, which has never certainly been observed to occur in the  $F_2$  generations grown from such naturally occurring hybrids.—C. A. SHULL, *The University of Chicago*.

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# CURRENT LITERATURE

## BOOK REVIEWS

### Permian floras of Asia

Two pretentious volumes have recently been issued by the geological surveys of the Soviet Union,<sup>1</sup> and of China.<sup>2</sup> They form a valuable supplement to some previously published volumes dealing with the carboniferous flora of Asia. Here may be mentioned the coal flora of Tonkin by R. ZEILLER, Paris, 1903; the flora of the coal basin of Heraclea in Asia Minor, by the same author, Paris, 1901; and the paleozoic flora of the Angara series by M. D. ZALESSKY, Petrograd, 1918.

The Permian flora of the Ouralian of the Angara continent is a continuation of ZALESSKY's earlier book, and both volumes, which are only atlases without an accompanying text but with introductions and explanations, deal with the late paleozoic vegetation of this peculiar continent, which extended eastward from what is now the Ouralian Mountains but which was at that time an archipelago. It is the classical ground of Permian geology, the Permian formation receiving its name from the city of Perm which is located on the west side of the Oural Mountains. South of the Angara continent was at that time a continent called Gondwana by the geologists. The Gondwana flora was characterized by *Glossopteris*, and Gondwana was later connected with the Angara continent. For this reason we find the Gondwana flora in the deposits of the Oural Mountains, but it is absent in Europe and North America.

ZALESSKY's atlas contains eight plates of the Permian plants from the Petchora basin of the Gondwana continent. It also contains numerous microphotographs of structural material obtained from the Permian of Siberia. The illustrations of plant habits show the similarity of numerous late paleozoic plants, especially pteridophytes in different parts of the world; but undoubtedly there was a well developed local flora on the Angara continent as far back as the mid-Carboniferous, and the theory of a completely uniform cosmopolitan flora all over the earth during the Upper Carboniferous will have to be partly revised.

The illustrations are excellent, and are a credit to the typographic facilities of the Soviet Union.

HALLE's book on the paleozoic plants from central Shansi describes and illustrates numerous fossils extending from the Stephanian to the Lower

<sup>1</sup> ZALESSKY, M. D., Permian flora of the Ural side of the Angara continent. Geol. Comm. U.S.S.R. Mem. New Series no. 176. Atlas Text in Russian and French. 4vo. pp. 52. pls. 46. Leningrad. 1927.

<sup>2</sup> HALLE, T. G., Paleozoic plants from central Shansi. Geol. Survey of China. Paleontologia Sinica. Series A. Vol. II. 4vo. pp. 316. pls. 64. 1927.

Permian, and possibly the Upper Permian. The flora of Shansi forms part of the Circumpolar Arctic carboniferous flora, and differs in its earlier phase from the contemporaneous floras of Europe and North America only about in the same degree as these differ from each other. The American relationship becomes somewhat more pronounced in the upper part, and at the same time types peculiar to eastern Asia increase in importance. The affinity to the *Glossopteris* flora and to the flora of Angara is very slight, and is only evident in the upper part of the plant-bearing series. The author characterizes this peculiar east Asiatic floral element with the name of "Gigantopteris" flora, and he concludes that the strong contrast between the Gigantopteris flora and the flora of Angara land can hardly be explained through the probable difference in age, but indicates the existence of two phytogeographic regions in Asia outside Gondwana land.

The illustrations, which have been made in Stockholm, are excellent, and in most cases permit the use of a magnifying glass. It is to be hoped that HALLE will soon issue other books based on the rich material which has been collected by Swedish and Chinese paleobotanists in China.—A. C. NOÉ.

#### Textbook of botany

A textbook of botany for college and university students has been prepared by ROBBINS and RICKETT\* which is distinctly different from the usual type of such books. The subject matter treated includes the conventional topics, but the point of view expressed and the method of attack are refreshing departures from the usual methods of presentation. Emphasis is placed on fundamental biological principles, the aim of science, and the scientific method rather than upon factual material, with the result that the book is not only readable, but introduces sufficient speculative material to challenge the attention of the beginning student in botany. The frankness with which the limitations of our knowledge regarding the causes of certain basic phenomena are admitted is praiseworthy; but, from the standpoint of pedagogy might possibly be expressed with more finesse in order to avoid the discouragement that the young student in botany is apt to feel at the abruptness of the dénouement.

The treatment of certain difficult sections is very satisfactorily handled in the first portion of the book, which deals with the functions and structure of the seed plant. The chapter on absorption of water and dissolved material is an excellent exposition of a difficult topic; and those on photosynthesis, transpiration, and the relations of living things to energy are adequately treated. The chapters on formation of new cells and growth might well be combined under the latter title, in order to emphasize more effectively the rôle of mitosis in growth processes. The sections on life and death and the origin of life are well presented, and the statements regarding vitalism and mechanistic theories in the former appeal to the writer as being sufficiently conservative.

\* ROBBINS, W. J., and RICKETT, H. W., Botany. 8vo. pp. xxiii+535. figs. 384. New York: D. Van Nostrand Co. Inc. 1929.

The latter portion of the book is designed to acquaint the student with the organization of the plant kingdom, the life processes and life cycles of representative members of each large group, and includes final chapters on inheritance, biologic evolution, and plant distribution. The sequence of chapters is a radical departure from the usual phylogenetic arrangement. Chapters on bacteria, yeasts, and fungi precede the section on the algae; and bryophytes are preceded by the chapter on ferns. The authors justify this novel treatment on pedagogical grounds. They state that the intimate connection between the fungi and the theory of spontaneous generation, as well as the new principles of physiology which the fungi illustrate, warrant the former change; and that "the fern is considered before the moss because of the clearer demonstration of alternation of generation given by its life history." This is an innovation which will receive considerable criticism from those teachers who are accustomed to the orthodox phylogenetic sequence; but the authors are to be commended for their attempt to improve the pedagogy in botanical writing. Certainly there is room for improvement, and experiments of this type should be given careful trial before judgments are made regarding the modification in question. The final sections, which include questions for review and references for collateral reading, should prove helpful to instructor and student alike.—H. E. HAYWARD.

#### Taxonomy

The first volume of the long awaited *Index londinensis*<sup>1</sup> has recently made its appearance. This work is the direct successor to the widely known and, to the taxonomist, invaluable *Iconum botanicarum index locupletissimus* of PRITZEL, first published in 1855. Nearly 84,000 entries are listed, drawn from upward of 4000 sources. It is stated that five further volumes will be issued and that the total number of entries will reach nearly 500,000. All species are arranged generically, and all genera are listed in alphabetical sequence, the last in this volume being *Campanopsis*. The primary purpose of this great undertaking is of course to afford immediate reference to all pictorial representations of the species, varieties, etc., of the higher plants. An attempt has been made to reach completeness for the post-Linnean period down to the end of the year 1920, but only a small number of pre-Linnean illustrations are cited. The value of a work like this to the monographer will be largely in direct proportion to the number of illustrations previously published in his own special field. Generally speaking, however, the *Index londinensis*, taken as a companion to the *Index kewensis*, will soon receive universal recognition as being well nigh indispensable to all workers in plant taxonomy and even to many in allied fields. The highest praise is merited by the Royal Horticultural Society of London, under whose auspices the volumes are being compiled, by Dr. STAFF, the honorary editor, and by a host

<sup>1</sup> STAFF, O., *Index londinensis* to illustrations of flowering plants, ferns and fern allies being an emended and enlarged edition continued up to the end of the year 1920 of PRITZEL's Alphabetical register of representations of flowering plants and ferns. pp. xx+547. Oxford: The Clarendon Press. 1929.



of institutions and individuals the world over who have generously collaborated in the vast amount of bibliographic work involved.

The seventh supplement to the *Index kewensis*<sup>1</sup> has reached American libraries. The need for a work of this type is at once apparent from the great activity revealed in the world's taxonomic centers. Thus, for example, while only a five-year period is covered, the genus *Aster* is credited with 31 new names, *Astragalus* 89, *Begonia* 57, *Drypetes* 110, *Hieracium* nearly 1000 (!), *Senecio* 105, etc. In the case of *Hieracium*, one notes with misgivings a remarkable diversity of authorship. It is hardly to be expected that if a multitude of workers attack independently the same genus at the same time their work can be free from numerous duplications. Students of general nomenclatural problems will be heartened to find a somewhat simpler nomenclature reflected throughout than prevails among the zoologists. The contrast in this respect seems even greater than is really the case, due of course to the unfortunate omission by the Kew authors of subspecific names. The scientific names are as a rule pronounceable. Some, however, seem hardly sensible, as, for example the name AA, adopted from the younger REICHENBACH by SCHLECHTER, for a genus of the Orchidaceae.—E. E. SHERFF.

#### Australian rain-forest trees

A recent volume<sup>2</sup> has, as its most notable feature, numerous halftone plates from photographs illustrating the bark and trunk characters of more than 100 species found in the humid tropical and semitropical forests of eastern Australia. The leaves and fruit are also shown from photographs which are mostly from herbarium material. The descriptions, which embrace some 150 species, contain many notes upon the appearance of the trees in the field, and should therefore be particularly useful for the recognition of species in the forest.

It is noticeable that these rain forests contain fewer endemic forms than do the semi-arid regions. Among the notable families represented by several to many species are the Fagaceae, Moraceae, Urticaceae, Lauraceae, Leguminosae, Rutaceae, Meliaceae, Euphorbiaceae, Myrtaceae, Sapotaceae, and Rubiaceae. Keys are provided for the recognition of families and genera.

There is a brief description of the rain forest itself, and notes on such things as the relation of the forests to the soil, to bush fires, and to rainfall distribution. The size of the trees, their bark characters, and the occurrence of buttressed bases are discussed and illustrated. While serving to give a general idea of areas of most interesting forests, the volume will find its greatest usefulness in field studies in Australia.—G. D. FULLER.

<sup>1</sup> Curators of Kew Herbarium, *Index kewensis plantarum phanerogamarum supplementum septimum nomina et synonyma omnium generum et specierum ab initio anni MDCCCXXI usque ad finem anni MDCCCXXV nonnulla etiam edita complectens*. pp. 260. Oxford: The Clarendon Press. 1929.

<sup>2</sup> FRANCIS, W. D., *Australian rain-forest trees; excluding the species confined to the tropics*. 8vo. pp. xi+347. pls. 213. figs. 25. *Rainfall map*. Brisbane: Anthony James Cumming, Government Printer. 1929. 10s.

## Recent advances in plant physiology

An attempt has been made by BARTON-WRIGHT<sup>1</sup> to summarize the recent developments in the field of plant physiology. The material is presented in interesting and attractive style, and the order of presentation seems logical. It follows almost the same sequence of presentation used for many years in courses in plant physiology at the Hull Botanical Laboratory.

The first chapter is devoted to soil and water relations of the plant, root system development, and physiological functions of roots. The second chapter presents the problems of transpiration, sap ascent, root pressure, etc., following which are chapters on photosynthesis, nitrogen metabolism, respiration, and the physiology of growth and reproduction. The student who desires to orient himself to the physiological phases of plant life, or the student of plant physiology, horticulture, agronomy, or forestry who desires a rapid review of the subject in its recent aspects, can survey it in a short time with this book, which is quite the best of its kind.

At certain places the reviewer found himself wishing that the text were more conducive to clear thinking on the part of the student; but this is hardly the author's fault, except that he has accepted ideas current with other authors. One of such parts of the book is that dealing with the absorption of water by roots (pp. 28-34). The use of the term "suction pressure" for the forcible entry of water into the cell is not appropriate, and the term "suction tension" proposed by BECK<sup>2</sup> more recently is little better. Surely there is no suction of any kind involved in the process, but rather an active, forcible diffusion of water into the cell against all the pressures that the cell exerts in the opposite direction (wall pressure, protoplasmic tension, etc.), because there is more free water outside than inside the cell. As the reviewer remarked in a paper presented before the American Society of Agronomy several years ago, if an automobile tire can be said to exert a "suction pressure" while being inflated with compressed air, then we can also properly speak of a "suction pressure" in cells, for water entry. The water forces its way into the cells, and is not sucked in by some partial vacuum formation, as the term implies.

The author has not tried to present a complete summary, but to select the papers which seemed to him most important. In this he has followed a wise course, for the mass of literature in plant physiology has grown to large proportions in the last two decades.

A whole essay could be written concerning certain points mentioned in the preface. To pass judgment whether plant physiology is less fundamental in America than elsewhere is more readily possible, the less experience or background one has for judging. Research, in order to be fundamental, must be

<sup>1</sup> BARTON-WRIGHT, E. C., Recent advances in plant physiology. 8vo. pp. 352. Philadelphia: P. Blakiston's Son and Co. 1930.

<sup>2</sup> BECK, W. A., Osmotic pressure, osmotic value and suction tension. *Plant Physiol.* 3:413-440. 1928.

fundamental to something that can be evaluated according to some standard. If it cannot be so judged, it should not be considered fundamental. This has been the main lack in some purely academic researches, which were at one time called fundamental because one could not see any possible application which might be derived from them, and from which nothing worth while ever has come.

The arts and sciences, such as horticulture, agronomy, forestry, bacteriology, and similar fields, must always include and have as a part of their secure foundation a large measure of botany. Instead of being in the "Cinderella" position in the botanical world, as BARTON-WRIGHT says, plant physiology is literally in the corner-stone position, and demands that its fundamental position be recognized. Without any thought of separation from other lines of botany, plant physiology proposes not to hide its light under a bushel, but to use every legitimate means to encourage and develop its service to the fields for which it does provide a foundation in practice.

The appearance of such books as this from time to time will help to stimulate an ever-widening interest in the problems of plant physiology, and will render less difficult the pathway of the student who must find some means of digesting, evaluating critically, and assimilating an enormous annual output of papers.—C. A. SHULL.

#### Flowers and flowering plants

The appearance of a new textbook of systematic botany<sup>1</sup> seems to indicate a revival of interest in that branch of plant science. It has as its special feature the system of classification elaborated by the late Professor BESSEY, whose portrait appears as a frontispiece in the volume. In using the Besseyan system as a foundation for his book, POOL has produced a fitting memorial to his great teacher. This will recommend the volume to all who knew and admired Professor BESSEY.

The material in the volume is well organized, beginning with a consideration of the anatomy of flowers, fruits, and seeds. The work of the flower is then considered, and much attention is devoted to pollination and to seed production and distribution. Floral diagrams and floral formulas are emphasized, and useful devices are suggested for representing floral structure in shorthand.

There is an excellent brief sketch of the history of the development of taxonomy, from the time of the herbalists to the ENGLER and the BESSEY systems. The advantages of the various systems are presented and the principles on which they are founded discussed.

The latter half of the book considers a characterization of selected orders and families. Here the descriptions are brief, and as simple as is compatible with accuracy. A notable feature of this part of the volume is the collection

<sup>1</sup> POOL, R. J., *Flowers and flowering plants. An introduction to the nature and work of flowers and the classification of flowering plants.* New York. pp. xx+378. figs. 191. New York: McGraw-Hill Book Co. 1929. \$3.50.

of drawings by F. SCHUYLER MATHEWS, illustrating members of the various families. It is a decided relief to have these familiar groups represented by a series of new, attractive, and accurate figures. There follows a chapter on collecting and preserving specimens, and a comprehensive list of reference books, monographs, and manuals.

The book is well written, and will form an excellent addition to the equipment of the teacher of elementary taxonomy. While the Besseyan system has many fine features to recommend it, its extensive adoption is somewhat doubted, even when so attractively presented as in this volume. The odds are too great in the accumulated mass of literature following the more widely known arrangement of ENGLER.—G. D. FULLER.

### NOTES FOR STUDENTS

Celluloid films from coal balls.—A method has been devised by KOOPMANS,<sup>2</sup> in collaboration with JOHN WALTON, to reproduce the surface of a coal ball section so accurately in a celluloid film that the latter can be used instead of a micro-preparation of the coal ball.

The method consists essentially of the following process. The cut surface of the coal ball is smoothed, and if it is a calcareous coal ball it is etched with hydrochloric acid; if siliceous, with hydrofluoric acid. After the surface has been dried a solution of celluloid is poured on it. As soon as the latter has become dry, the membrane of celluloid is pulled off and either preserved or mounted in Canada balsam.

KOOPMANS states that the surface should not be highly polished, but merely smoothed; otherwise the etching takes too long. The etching must be continued until the moist surface shows organic remains in relief. It is important to establish the time necessary for etching and concentrating the acid by experiment, since these factors will vary with different coal balls. The superfluous acid should be removed by dipping the preparation into water, not by pouring water on it. In drying, the etched surface should not be touched by hand; also excessive heating should be avoided since the organic remains on the etched surface are easily burned. When the celluloid solution is poured upon the etched surface the latter must be as horizontal as possible, and should be moistened with amylacetate to avoid the formation of air bubbles in the celluloid film. The solution of celluloid in amylacetate may be made from cleaned photographic films, and should have the consistency of syrup. After the celluloid solution has been poured on, the stone should be left in a dustfree place for drying, which may take 24-48 hours. In order to remove the celluloid film it is advisable to cut the etch with a sharp knife and to slip the latter between the celluloid membrane and the stone. The film is pulled off slowly, and should be dipped in a thin solution of chloric acid to remove any particles of rock previous to

<sup>2</sup> KOOPMANS, R. G., Yearbook for 1928. Geol. Bur. Netherlands, Heerlen. 131-132. 1929.

preserving dry or imbedding in Canada balsam. Before making another film it is necessary to repolish and re-etch the coal ball.

The advantages of the new method are obvious. It is easy to make about ten preparations per 1 mm. thickness of the coal ball, and to obtain in this way consecutive series of thin sections of seeds or sporangia which could not be obtained in any other way. It is also easy to make large preparations, while the preparing of very large thin sections of coal balls is a highly technical, difficult, and expensive process. The most obvious advantage is the great saving of time, since the preparation of a celluloid film takes only 10-15 minutes, while the cutting, grinding, polishing, and mounting of a thin section consumes 2-3 hours.—A. C. NOÉ.

**Micropaleobotany.**—This paper<sup>1</sup> is project no. 3 in the research program of the American Petroleum Institute. Its first part, by STADNICHENKO, contains preliminary results of studies of supposed "mother rocks" of petroleum by means of a micro-furnace; and the second part, by WHITE, gives a description of the fossil plants found in these mother rocks. The latter includes (1) a rich boghead of algal coal; (2) a sedimentary rock made up almost entirely of the exines of very large spores; (3) a brown oil shale containing many spore exines, a number of which are twisted; (4) a marine deposit of the general structure and characters of an ordinary oil or bituminous shale. Specimens listed under 1-3 are probably Lower Cretaceous, while specimen 4 is Triassic.

The fossil plants contained in the "mother rocks" were described by WHITE under the following names, all of which represent new species: *Reinschia alaskana* D. W.; *Sporangites alaskensis* D. W.; *S. arctica* D. W. *R. alaskana* is a large one-celled alga, forming flattened colonies, with enormously fatty, thickened walls, and is in close agreement as to size, arrangement, and cell details with the "yellow bodies" in the bogheads from the Colville River basin, Alaska, illustrated by THIESSEN in his paper on the origin of the boghead coals.<sup>2</sup> THIESSEN has shown the algal nature of *Reinschia* contrary to the earlier assertions of some paleobotanists. The two species *Sporangites alaskensis* and *S. arctica* are based on spore exines. It is to be hoped that more of such interesting investigations will be published in the near future, on account of the light they throw, not only upon the origin of petroleum, but also upon the algal forms of the Paleozoic and Mesozoic floras.—A. C. NOÉ.

**Fossil flora of Aachen.**—The Geological Survey of Holland has added another valuable paleontological and stratigraphical monograph<sup>3</sup> to the series of

<sup>1</sup> STADNICHENKO, T., Microthermal studies of some "mother rocks" of petroleum from Alaska. WHITE, D., Description of the fossil plants. Bull. Amer. Assoc. Petroleum Geologists 13:823-848. 1929.

<sup>2</sup> U.S. Geol. Survey Prof. Paper 132. 121-138. 1925.

<sup>3</sup> DE VOOGE, N., Gliederung und Fossilführung des tieferen Oberkarbons in der Umgebung von Aachen und den angrenzenden Gebieten von Holland und Belgien. Geologisch Bureau voor het Nederlandsche Mijngedee te Heerlen. Jaarverslag over 1928. Heerlen, pp. 11-72. Charts 1-4. pls. 1-5. figs. 1-14. 1929.

its publications. While considerable attention is naturally paid to purely stratigraphical and paleozoological items, much paleobotanical material is included, especially its application to the stratigraphy of the coal basin of Aachen and its correlation with the coal seams of other basins.

Many contributions have been made to paleobotany and coal geology in the publications of the Dutch Geological Bureau, especially by JONGMANS,<sup>1</sup> who contributed to the same volume in which DE VOOGD's monograph appears. Other articles of interest for paleobotanists in this volume are by KOOPMANS<sup>2</sup> and LEBEDEW.<sup>3</sup>—A. C. NOÉ.

**Elongation of mesocotyl in oats.**—Research workers, experimenting with oats in connection with stimulus responses such as tropisms, have often encountered considerable difficulty in obtaining vertical coleoptiles. They have usually obtained a high percentage of bent oats, due to an undesirable elongation of the mesocotyl. BEYER<sup>4</sup> accidentally obtained 100 per cent straight coleoptiles, and on considering the matter found that he had unwittingly left the germinating seeds for the first two days in the greenhouse exposed to diffuse light. In a series of preliminary tests he found that the presence of light was the main factor in repressing elongation of the coleoptile, although additional factors were low temperature and the lowering of soil moisture.

Subsequent investigations on the effect of light are reported by LANGE,<sup>5</sup> in a series of experiments, with abundant data to support his conclusions. Germinations carried on in darkness, diffuse, and bright daylight showed progressively shorter mesocotyls. Weak red light, acting similarly to white light of low intensity, revealed the fact that the greatest effect was obtained when exposure was made during the last 12-24 hours of the total 48 hours during which swelling of the seed takes place. Some effect is also obtained if the exposure occurs during the first 24 hours of this period, which is the more surprising when one considers that the light must at that time penetrate not only the glumes but the seed coat as well.—THORA M. PLITT.

<sup>1</sup> JONGMANS, W. J., *Algemeene Bouw van het Limburgsche Karboon*. II. *ibid.* pp. 73-130. Chart 5.

<sup>2</sup> KOOPMANS, R. G., *Celluloidpreparat anstatt Dünnschliff*. *ibid.* pp. 131-132.

<sup>3</sup> LEBEDEW, N., *Über die Namurstufe in Donetzbecken*. *ibid.* pp. 133-134.

<sup>4</sup> BEYER, A., *Zur Keimungsphysiologie von *Avena sativa**. *Ber. Deutsch. Bot. Ges.* 45:179-187. 1927.

<sup>5</sup> LANGE, S., *Über den Einfluss weissen und roten Lichtes auf die Entwicklung des Mesocotyls bei Haferkeimlingen*. *Jahrb. Wiss. Bot.* 71:1-25. 1929.

# THE BOTANICAL GAZETTE

June 1930

## REVISION OF NORTH AMERICAN SPECIES OF GODETIA

C. LEO HITCHCOCK

(WITH ONE FIGURE)

### Introduction

This study, which has been carried on at the Herbarium of Pomona College, was undertaken at the suggestion of Dr. PHILIP A. MUNZ. He had collected important notes on the genus *Godetia* while at the Gray Herbarium, and I am deeply indebted to him for the use of these notes, as well as for his guidance in the preparation of this paper.

In the summer of 1928 the herbarium of the California Academy of Sciences was visited, with access to valuable notes on the genus by Miss ALICE EASTWOOD, to whom gratitude is hereby expressed. I wish also to thank Dr. W. A. SETCHELL of the University of California for the loan of the important memoranda on *Godetia* left by the late Mrs. KATHARINE BRANDEGEE, whose knowledge of the group cannot be questioned. I am indebted also to several others who have either furnished color notes, or looked up references. They are Mr. T. A. SPRAGUE of the Royal Botanic Garden at Kew, Mr. DAVID KECK of the Carnegie Institute, Dr. AVEN NELSON of the University of Wyoming, Professor P. B. KENNEDY of the University of California, and Mr. THOMAS CRAIG of Pomona College.

For carrying on this revision, material has been available from the following sources: Pomona College Herbarium (P), Herbarium of the University of California (C), Gray Herbarium of Harvard Uni-

versity (G), Dudley Herbarium of Stanford University (S), Herbarium of the Santa Barbara Museum (SB), private herbarium of Dr. JEPSON (Jep). To the curators of these herbaria, and particularly to Dr. W. L. JEPSON, who very kindly lent his types, I wish to express appreciation. The abbreviations indicated for the different herbaria are those used in this paper when citing specimens.

### History of genus

The first species of *Godetia* was published by CURTIS, Bot. Mag. 10: pl. 352. 1796, as *Oenothera purpurea*; it was described from material grown in the Royal Garden at Kew, from seeds collected on the western coast of North America. The next two species published, *Oe. tenella* Cav. Ic. 4:66, pl. 396. 1797, and *Oe. tenuifolia* Cav. loc. cit. 67, pl. 397, were South American. After these followed *Oe. romanzowii* Ledeb. ex Hornem., Hort. Hafn. Suppl., 133. 1819; *Oe. amoena* Lehmann, Ind. Sem. Hort. Hamb. 8. 1821; *Oe. roseo-alba* Bernh., Ind. Sem. Hort. Erford (1824); ex Reichb. Ic. Bot. Exot. 1:34, pl. 47. 1827; *Oe. quadrivulnera* Douglas in Lindl., Bot. Reg. 13: pl. 1119. 1828; *Oe. viminea* Dougl. ex Hooker in Bot. Mag. et *Oe. lindleyi* Dougl. in Bot. Mag. 55: pl. 2832. 1828; loc. cit. pl. 2873; and *Oe. decumbens* Dougl. in Bot. Mag. 56: pl. 2889. 1829. Five of these species were treated by SERINGE in DC. Prodr. 3:48-49. 1828.

*Godetia* was proposed as a genus in 1835 by SPACH, Hist. Nat. Veg. Phan. 4:386-392. 1835, being named for Mr. CHARLES GODET, a Swiss botanist; here eight species were recognized. That same year SPACH published a more scientific treatment, Nouv. Ann. Mus. Par. III, 4:387-394. 1835, where he recognized eleven species; eight of them, *G. willdenowiana*, *G. decumbens*, *G. viminea*, *G. quadrivulnera*, *G. romanzowii*, *G. lehmanniana*, *G. lindleyana*, and *G. bottae* from North America; the other three, *G. tenuifolia*, *G. gayana*, and *G. cavanillesii* (*tenella*) from South America. HOOKER, Fl. Bor. Am. 1:209-213. 1840, and TORREY and GRAY, Fl. N. Am. 1:502-505. 1840, did not recognize *Godetia* as a genus. The last attempt to include both North and South American species in one treatment was by WALPERS, Repertorium 2:87-89. 1843, where *Godetia* was made to include seventeen species.

In the following references, only North American species have



been considered. WATSON, Proc. Am. Acad. 8:596-600. 1873, followed TORREY and GRAY in treating *Godetia* as a section under *Oenothera*; he recognized fourteen species: *G. grandiflora*, *G. purpurea*, *G. lepida*, *G. albescens*, *G. williamsonii*, *G. quadrivulnera*, *G. tenella*, *G. viminea*, *G. romanzowii*, *G. amoena*, *G. bottae*, *G. epilobioides*, *G. hispidula*, and *G. biloba*; but later, Bot. Cal. 1:228-231. 1876, he recognized *Godetia* as a genus. The only modern revision of the group was by JEPSON, Univ. Cal. Pub. Bot. 2:319-354. 1907, who had seventeen species and many varieties. LEVEILLE included the group in his monograph in 1908, using four species and eleven varieties. NELSON and MACBRIDE, BOT. GAZ. 65:59-65. 1918, transferred the genus to *Clarkia*, scarcely changing JEPSON's concept of species and varieties. In addition to these more inclusive references, many others could be cited where single species have been proposed. Altogether about fifty species have been described, eight of which were South American. For North America I am recognizing twelve species and eleven varieties.

#### Generic status and relationship

NELSON and MACBRIDE, BOT. GAZ. 65:59-60. 1918, called attention to the fact that *Godetia* and *Clarkia* are perhaps too closely related to be kept as distinct genera. In order to describe the generic status of *Godetia* more authoritatively, Dr. P. A. MUNZ and I have collaborated in a study of the species commonly included in *Clarkia* and *Eucharidium*. It has been decided to keep *Godetia* as a separate genus (Bull. Torr. Club 56:181. 1929).

Because of lack of space, relationships within the genus are not discussed, other than those indicated in fig. 1.

#### *Godetia* Spach

GODETIA AS A GENUS: Spach Hist. Nat. Veg. Phan. 4:386. 1835, Nouv. Ann. Mus. Par. III, 4:387. 1835; Walp. Rep. 2:87. 1843; Greene Fl. Fran. 219. 1891; Raimann in Engl. and Prantl Nat. Pflfam. 3, Ab. 7:213. 1898; Jepson, Univ. Cal. Pub. Bot. 2:324. 1907; Piper and Beattie Fl. N. W. Coast 250. 1915; Jepson Man. Fl. Pl. Cal. 674. 1925.

GODETIA AS A SECTION: T. and G. Fl. N. Am. 1:502. 1840; Watson, Proc. Am. Acad. 8:596. 1873; Leveille Monog. Onoth. 260. 1908.

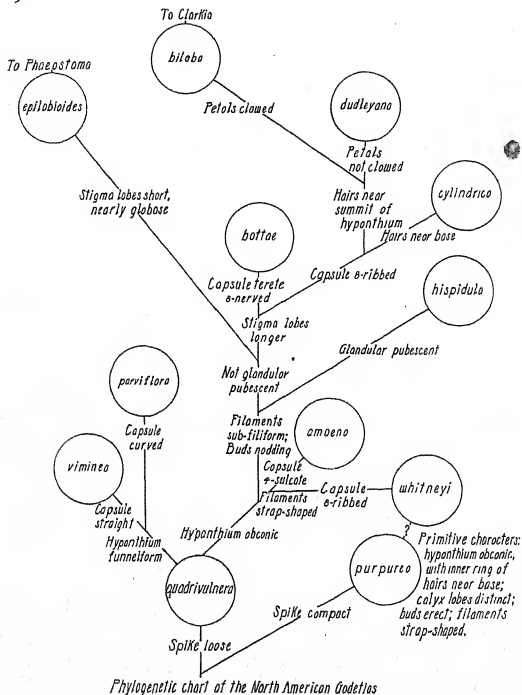


FIG. 1

GODETIA INCLUDED IN CLARKIA: Nels. and Macbr., Bot. Gaz. 65:59. 1918.

Calyx tube obconic to narrowly funnellform, with inner ring of hair. Calyx lobes distinct and reflexed, or partially or wholly united and turned to one side. Stamens in two series, those opposite petals shorter; filaments filiform to flattened; anthers subequal to equal, usually wholly fertile, but tips sometimes sterile, sparsely hairy. Stigma lobes short, ovoid to linear, yellow to purple. Capsules 4-sulcate, terete and 8-nerved, or heavily 8-ribbed, linear to ovoid, sessile to long pedicelled, beakless to long beaked. Seeds brown, sometimes somewhat cellular-puberulent, upper margin fimbriate. Flowers white to purple; petals cuneate to obovate, apex entire, erose, retuse or bilobed, clawless or with claw as much as 2 mm. long. Buds nodding or erect. Inflorescence a spike or small panicle, usually loose, but sometimes much shortened. Leaves linear to spatulate, lower ones commonly deciduous, reduced in size up the stem, born in fascicles, the secondary ones much reduced in size, usually remotely denticulate, sometimes entire. Epidermis of stems exfoliating below. Annuals.

**Type species:** *G. purpurea*.

The type species is rather difficult to determine by ordinary criteria. From SPACH's description apparently he had no particular species in mind when proposing the genus; however, *G. purpurea* (*G. willdenowiana*) is the first species listed by him and, furthermore, is the first of the species now included in *Godetia* which was made known to science.

#### Key to species

- A. Hypanthium with inner ring of hairs one-fourth to three-fifths way from base to summit; buds erect except in nos. 3, 7, and 8.
  - B. Stigma lobes 4-7 mm. long.
    - C. Capsule greatly enlarged at middle; petals 4-6 cm. long. Shelter Cove, Humboldt Co., California. . . . 4. *G. whitneyi*
    - C. Capsule not greatly enlarged at middle; petals not over 4 cm. long. Watsonville, California, to British Columbia  
3. *G. amoena*
  - B. Stigma lobes less than 4 mm. long.
    - C. Capsule 4-sulcate when immature, terete and 8-nerved when dried. . . . . 3. *G. amoena*

C. Capsule 8-ribbed when immature, terete or somewhat quadrilateral and 8-nerved when dried.

D. Buds nodding; calyx lobes united in anthesis.

E. Capsules elongate, linear, frequently quadrilateral and smooth in mature specimens; petals lavender, usually with purple base; stigma lobes oval; inflorescence never glandular-pubescent. Plants of inner Coast Ranges, from Los Angeles Co. to Fresno Co., California

8. *G. cylindrica*

E. Capsule not linear, ribs evident in dried material; petals lavender without purple base; stigma lobes linear; inflorescence frequently glandular-pubescent. Plants of Sierran foothills from Mariposa Co. to Butte Co., California.....7. *G. hispidula*

D. Buds erect; calyx lobes usually distinct, but sometimes united, especially in no. 6 and one variety of no. 1.

E. Hypanthium 5-15 mm. long, slender toward base, flaring at top; ovary usually with annular swelling at summit.

F. Plants branching from base, somewhat ascending; branchlets filiform; capsule usually quite strongly curved. Coastal plants from San Luis Obispo Co. to Santa Cruz Co., California.....6. *G. parviflora*

F. Plants branching mainly from middle, erect; branchlets stout; capsule straight, or nearly so. Plants chiefly from Sierras and region about San Francisco Bay

5. *G. viminea*

E. Hypanthium 2-7 mm. long, tapered uniformly from base to summit; without annular swelling at top of ovary.

F. Inflorescence congested in normal plants; capsule enlarged at middle; leaves 3-18 mm. wide. Plants usually found at edge of lakes or drying pools

2. *G. purpurea*

F. Inflorescence not congested in normal plants; capsules not enlarged at center; leaves 2-8 mm. wide. Plants usually found in rather dry or well-drained soil.....1. *G. quadrivulnera*

- A. Hypanthium with inner ring of hairs at least three-fifths of way from base to summit; buds nodding.
- B. Petals less than 1.5 cm. long, white or cream, sometimes tinged with pink; stigma lobes very short, not over 0.5 mm. long. Chiefly Southern California.....11. *G. epilobioides*
- B. Petals 1.5 cm. or more long, lavender to purple; stigma lobes more than 0.5 mm. long.
- C. Capsule narrowly linear, 2-4 cm. long, 0.1-0.2 cm. thick; petals lavender with purple base. Along inner Coast Ranges from Los Angeles Co. to Fresno Co., California
8. *G. cylindrica*
- C. Capsule thicker; petals lavender but without purple base.
- D. Filaments unequal, slightly flattened; capsule terete and very faintly nerved in living material, quadrilateral and obscurely nerved or smooth when mature, especially in herbarium material; pedicels as much as 2 cm. long. Near coast from Orange Co. to Monterey Co., California
9. *G. bottae*
- D. Filaments subequal, subfiliform; capsule 8-ribbed in living material, ribs very evident when mature or dried; pedicel usually less than 3 mm. long.
- E. Petals bilobed; capsule short-pedicelled. Sierra Nevada foothills from Mariposa Co. to Nevada Co., and from Contra Costa Co., California.....12. *G. biloba*
- E. Petals not bilobed; capsule sessile to nearly so. Mountains from San Jacinto Range northward through Sierras to Mariposa Co., California.....10. *G. dudleyana*

#### Treatment of species

1. GODETIA QUADRIVULNERA (Dougl.) Spach Hist. Veg. Phan. 4: 389. 1835.

Plants usually erect, sometimes ascending, branching from base or middle, stems 1-8 dm. tall; leaf blades lanceolate to spatulate, 1-5 cm. long, 0.2-0.8 cm. wide, acute to rounded, short petioled or sessile; buds erect; hypanthium 2-6 mm. long, brownish-green outside and within, inner ring of hairs about one-third way from base; calyx lobes green or yellow, 5-10 mm. long, usually distinct and well reflexed in anthesis, but sometimes united in pairs, or all united, the

tips not free in bud; petals varying from pale lavender, with or without spot of purple at apex or in center, to deep purple, 0.5-2 cm. long, 0.5-1.8 cm. wide, cuneate, the apex rounded to truncate and usually somewhat erose, not clawed; filaments unequal, 1-6 mm. long, the short ones one-third to one-half as long as others; anthers subequal, 2-4 mm. long, white to pale lavender; style varying in length from scarcely equal to short stamens to slightly longer than long ones; stigma lobes about 1 mm. long and as broad, lavender to purple; ovaries sparsely pubescent to densely white- or brown-hirsute, about equal to length of buds; capsules 1-3.5 cm. long, 2-3 mm. thick, not noticeably enlarged at center, sessile or with pedicels as much as 2 mm. long, tapering to beak 0.5-3 mm. long, terete and 8-ribbed with a faint nerve between ribs in fresh material, the capsules in dried specimens often square in cross-section, this squareness being accentuated on pressing, at which time nerves between the ribs are quite conspicuous; seeds 1 mm. long and broad, cresting about one-fifth as long as seed proper.

#### Key to varieties

- A. Leaves short, 1-2 cm. long, oblong to spatulate, rounded; capsules scarcely beaked, ribs very prominent, especially along sutures. Coastal, from Monterey Co. to Humboldt Co., California.....1a. *G. quadrivulnera* var. *davyi*
- A. Leaves longer, 2-5 cm. long, elliptic, acute; capsules blunt to long-beaked, ribs about equal in prominence.
  - B. Calyx lobes usually united; capsule very slender, with beak 2-4 mm. long. Chiefly from Santa Clara and Sacramento Valleys, California.....1b. *G. quadrivulnera* var. *vacensis*
  - B. Calyx lobes usually reflexed and distinct; capsule somewhat stout, beak usually not over 2 mm. long. Lower California to Washington.....1c. *G. quadrivulnera* var. *typica*
- 1a. *GODETIA QUADRIVULNERA* var. *DAVYI* Jepson, Univ. Cal. Pub. Bot. 2:341. 1907.—*Oenothera tenella* Wats., Proc. Am. Acad. 8:615. 1873, as to Monterey, *State Survey* 638, collection; *Godetia parviflora* Jepson, *loc. cit.* 339, as to Monterey, *State Survey* 638, coll.

Leaves oblong to spatulate, 1-2 cm. long, 0.3-0.6 cm. wide, rounded; calyx lobes reflexed in pairs or distinct; capsule 1-2.5 cm. long,

not beaked, finely and rather sparsely white-pubescent, ribs glabrous, brown, very prominent, especially at the sutures.

Type locality, Point Reyes, Marin Co., California.

Material examined, CALIFORNIA: Monterey, *Parry* (C), *Abbott* in 1904 (S), *Brewer* 638 (C), *Guirardo* 638 (G); Santa Cruz Co., Santa Cruz, *Jones* in 1881 (P); San Mateo Co., Ocean View, *Heller* 8382 (C,G,S), *Heller* 8384 (G,S), *Congdon* in 1889 (G), *Walker* 1142 (C); Marin Co., Pt. Reyes, *Davy* 6701, type collection (C), *Elmer* 4610 (C,P,S); Mt. Vision, *Hall* 8511 (C); Humboldt Co., Humboldt Bay, *Tracy* 2592 (C,G), *Tracy* 3127 and 3256 (C); Alton, *Tracy* 3771 and 3772 (C); Kneeland Prairie, *Tracy* 3030 (C); Del Norte Co., Lake Earl, *Parks* 8276 (C).

1b. *GODETIA QUADRIVULNERA* var. *VACENSIS* Jepson, Univ. Cal. Pub. Bot. 2:341. 1907. Leaves lanceolate, 2–5 cm. long, 0.2–0.4 cm. wide, acute; calyx lobes usually united in anthesis; capsule 2–4 cm. long, very slender, beak 2–4 mm. long, ribs of equal prominence.

Type locality, Vaca Mts., Napa or Solano Co., California.

Representative material, CALIFORNIA: Ventura Co., Ojai Valley, *Hall* 3192 (C); San Luis Obispo Co., Paso Robles, *Cobb* in 1907 (C); Monterey Co., Santa Lucia Mts., *Barber* in 1899 (C); 10 mi. N. of Bradley, *Mallory* in 1920 (S); Santa Cruz Co., below Big Trees, *Dudley* in 1893 (S); Santa Clara Co., Cupertino, *Heller* 8552 (G,S); Tuff Hill, *Cameron* in 1900 (S); Stanford University, *Atkinson* in 1900 (S); San Benito Co., Griswold Creek, *Abrams* and *Borthwick* 7944 (S); Hernandez, *Dudley* in 1899 (S); Eagle Creek, *Dudley* in 1899 (S); Contra Costa Co., Vallejo Junction, *Michener* and *Bioletti* 177 (G); Solano Co., near Vacaville, *Jepson* in 1891, probably typical material (S); Sacramento Co., Arbuckle, *King* in 1905 (C). The following are more or less intermediate between this variety and var. *typica*: Sierra Valley, Rattan (S); Shasta Co., Redding, *Heller* 7902, type collection of *G. rostrata* Eastw. *Muhlenbergia* 2:103. 1905, *nomen nudum* (G); Yolo Co., Winters, *Heller* and *Brown* 5584 (G,S); Mt. Diablo, *Abrams* 5712 (S); Stanford University, *Baker* 843 (C,G,P,S); *Geol. Surv. Cal.* (G).

1c. *GODETIA QUADRIVULNERA* var. *typica*, nom. nov.—*Oenothera quadrivulnera* Dougl. ex Lindl., Bot. Reg. 13:t. 1119. 1828; T. and G. Fl. N. Am. 1:504. 1840; S. Wats., Proc. Am. Acad. 8:598. 1873; *Godetia quadrivulnera* Spach, loc. cit.; Jepson, Univ. Cal. Pub. Bot. 2:340. 1907; *Clarkia quadrivulnera* Nels. and Macbr., Bot. Gaz. 65: 63. 1918; *Oe. quadrivulnera* var. *hirsuta* Kellogg, Proc. Cal. Acad. 5: 45. 1873; Curran, Bull. Calif. Acad. 1:137. 1885; *G. quadrivulnera* var. *tenella* Jepson Fl. W. Mid. Calif. 334. 1901; *G. quadrivulnera*

var. *apiculata* Jepson, Univ. Cal. Pub. Bot. 2:341. 1907; *G. quadrivulnera* f. *flagellata* Jepson, loc. cit.; *G. quadrivulnera* var. *flagellata* Jepson Man. Fl. Pl. Cal., 678. 1925; *G. quadrivulnera* f. *setchelliana* Jepson, Univ. Cal. Pub. Bot. 2:341. 1907; *G. quadrivulnera* var. *setchelliana* Jepson Man. Fl. Pl. Cal., 678. 1925; *G. quadrivulnera* var. *hallii* Jepson, Univ. Cal. Pub. Bot. 2:341. 1907; *G. quadrivulnera* var. *rubrissima* Jepson, loc. cit. 342; *Oe. prismatica* var. *quadrivulnera* Leveille Monog. Onoth. 267. 1908; *Oe. viminea* var. *intermedia* Kellogg, Proc. Cal. Acad. 1:61. 1854-57, Curran, Bull. Cal. Acad. 1:137. 1885; *Oe. tenella* Wats., Proc. Am. Acad. 8:598. 1873, in part; *Oe. auricula* var. *tenella* Leveille Monog. Onoth. 270. 1908, in part; *Godetia bingensis* Suksd., Deutsch. Bot. Monat. 18:88. 1900; *G. albescens* var. *micropetala* Jepson Fl. W. Mid. Cal. 334. 1901; *G. goddardii* Jepson, Univ. Cal. Pub. Bot. 2:342. 1907; *G. goddardii* var. *miguelita* Jepson, loc. cit.; *G. sparsifolia* Jepson, loc. cit. 347.

Leaves oblong to lanceolate, 2-5 cm. long, 0.2-0.6 cm. wide, acute; calyx lobes usually distinct in anthesis; capsule varying, beak not usually over 2 mm. long, ribs of equal prominence.

Type locality, "North West of North America."

Representative material, WASHINGTON: Klickitat Co., Bingen, *Suksdorf* 86, isotype of *G. bingensis* (G,S); Clallam Co., Olympic Mts., *Elmer* 2567, isotype of *G. brevistyla* (S). OREGON: Josephine Co., W. fork of Illinois R., *Abrams* 8639 (P,S); Grant's Pass, *Prescott* in 1912 (G,S); Marion Co., Salem, *Nelson* 727, flowers unusually large, (S); Hood River Co., Lower Hood River, *Henderson* 835 in 1924 (G). CALIFORNIA: without locality, *Douglas*, probably type material (G); Howell Mt., *Jepson* 2441, type of *G. quadrivulnera* var. *apiculata* (Jep); Klamath Hills, *Butler* 1375 (C,P); Trinity Co., Rush Creek, *Yates* 405 (C); Humboldt Co., Redwood Forest, *Abrams* 6028 (S); Mendocino Co., Ukiah, *Munz* 9867 (P); Glenn Co., near Bennett Spring, *Heller* 11540 (C,G,S); Butte Co., Cohasset, *Heller* 11908 (G,S); Plumas Co., between Taylorville and Crescent Mills, *Heller* and *Kennedy* 8827 (C,G,S); El Dorado Co., New York Ravine, *Brandegee* in 1907 (C); Amador Co., Jackson, *C. L. Hitchcock* 30 (P); Pine Grove, *Hansen* 1157 (P,S); Calaveras Co., Copperopolis, *Davy* 1337 (C); Tuolumne Co., Bear Creek, *Mrs. Williamson* 58 (P,S); Sonoma Co., Petaluma, *Kellogg* in 1870, type material *G. quadrivulnera* var. *hirsuta* (G); Lake Co., near Lakeport, *Baker* 3060 (C,G,P); Napa Co., Vaca Mts., *Jepson*, June 12, 1893, type *G. quadrivulnera* f. *flagellata* (Jep, C); Contra Costa Co., Mt. Diablo, *Abrams* 8040 and 8048 (S); Alameda Co., Alameda, *Bolander* in 1866 (G,C), *Dr. Kellogg* in 1866, type material *G. viminea* var. *intermedia* (C); San Mateo Co., Big Basin, *Dudley* in 1897 (S); Santa Clara Co., near Stanford University,



Baker 5066 (C,P), 859 (G,P); Mt. Hamilton, Elmer 4825 (C,P,S); San Benito Co., Hernandez, Lathrop in 1903 (S); Santa Cruz Co., Santa Cruz Mts., Jepson 13335, type of *G. quadrivulnera* f. *setchelliana* (Jep); Monterey Co., near Del Monte, Heller 6834 (G,S); near seaside, Heller 6754, approaching var. *davyi* (C,G,P,S); San Miguelito Rancho, Jepson 1625, type *G. goddardii* var. *miguelita* (Jep); San Luis Obispo Co., San Luis Obispo, Brewer 477 (C,G); Cuesta Pass, C. L. Hitchcock 7 (P); Price Canyon, C. L. Hitchcock 3 (P); Atascadero, Brewer 508 (C); Santa Barbara Co., Knapp's Lodge, Hoffman in 1925 (SB); road to Montecito, Eastwood 151 (C,G); Ventura Co., Nordhoff, Hall 3194 (C); Los Angeles Co., Saugus, K. Brandegee in 1909 (C); Claremont, Baker 5352 (P,S); Palmer's Canyon, near Claremont, C. L. Hitchcock 54 (P); Sepulveda Canyon, Santa Monica Mts., Abrams 304 (S); Santa Catalina Isl., Dunkle 1870 (P); San Bernardino Co., Potato Canyon, Parish 3222 (G,S); Mojave River, Palmer 141 (C), Parish 4987 (S); Arrowhead Springs, Feudge 1562 (P); Riverside Co., east base Box Springs Mts., Hall 6240, type collection *G. quadrivulnera* var. *rubrissima* (C,P), Hall 6241, type collection *G. quadrivulnera* var. *hallii* (C,P); San Jacinto Mt., S. B. and W. F. Parish 1501 (G,S); San Diego Co., San Diego, Thurber 533 (G), Palmer 104 (G); Jacumba, Abrams 3688 (G,S); Palomar Mts., Munz 8217 (P); Descanso, K. Brandegee in 1906 (C), Munz and Harwood 7172 (P). LOWER CALIFORNIA: North of Ensenada, Canby in 1925 (P).

*Godetia quadrivulnera* is the most widespread, as well as the most variable species of the genus. There seems to be no constancy in the coloration of petals; plants with flowers varying from pale lavender to purple, with or without spots, are often found growing together, all apparently from the same seed. Because of this fact I do not consider it wise to recognize such varieties as *hallii* and *rubrissima*, which are merely color variations. The pubescence of the inflorescence also shows extreme variation, many collections showing plants which have some capsules that are densely hirsute and others that are merely strigillose. Therefore, there is no reason for recognizing Kellogg's variety *hirsuta*. Other collections with hirsute capsules are: Santa Lucia Mts., K. Brandegee in 1909 (C); San Luis Obispo, Brewer 477 (C,G).

No difference can be seen between the type material of *G. bin-gensis* and ordinary *G. quadrivulnera*. Likewise *G. brevistyla* is not recognized, since style length is a rather variable character in *Godetia*, and much of the material of *G. quadrivulnera* from California has just as short styles as has *G. brevistyla*. *G. goddardii* I consider to be a rather large-flowered form of *G. quadrivulnera*; the

spike is somewhat shortened, but not unusually so, and the capsules are certainly those of *G. quadrivulnera* (1.5 cm. long, 8-ribbed, puberulent, and not thickened at the middle). *G. goddardii* var. *miguelita* has rather short, thick capsules, but they are no more hairy than much of the material from the Monterey region. The flower is small, being undoubtedly that of *G. quadrivulnera*, and the inflorescence is a rather loose spike, with the capsules 1.5-5 cm. apart. *G. quadrivulnera* var. *flagellata* is not unusual. It is a tall plant with the capsules well scattered. I believe, however, that this condition is largely ecological; certainly the inflorescence of *G. quadrivulnera* becomes shortened where the plants grow in a dry, poor soil. *G. quadrivulnera* var. *setchelliana* is not different from ordinary material, so far as can be seen; in the type collection one of the plants shows basal branching, but two others, somewhat smaller, are unbranched, and the young ovaries of almost any collection are as canescent as those of this collection. The var. *apiculata* is a rather slender plant, with slender capsules and buds, the latter having an unusually long tip, but this does not warrant nomenclatural recognition.

*G. quadrivulnera* is very easily confused with *G. purpurea* and *G. viminea*. Where the former species grows in poor soil, or lacks sufficient moisture, the inflorescence tends to become shortened and the flowers crowded, consequently the material resembles *G. purpurea*. It was such a form from Tracy, San Joaquin Co., Cobb in 1900 (C), that JEPSON called *G. sparsifolia*. Other collections of this nature are: Calaveras Co., Copperopolis, Davy 1367 (C); San Diego Co., Lakeside, Brandegee in 1906 (C); El Dorado Co., Simpson's Ranch, Sweetwater Creek, K. Brandegee in 1908 (C); Scott River Valley, Gilbert in 1899 (C). In general, however, *G. purpurea* has wider leaves and a more inflated capsule than *G. quadrivulnera*. Some robust specimens of *G. quadrivulnera* have petals as large as those of *G. viminea*, examples being: Griffith Park, Los Angeles, Braunton 401 (C); San Bernardino Co., Cucamonga Canyon, Williams (C); Humboldt Co., Hupa Road near Redwood Creek, Jepson 1971, type of *G. goddardii* (Jep); Santa Ana River Canyon, Munz, Street, and Williams 2669 (P,S); Marin Co., hills above Fairfax, K. Brandegee (C). The only methods of distinguishing such plants from *G. viminea*

are by the stigma lobes, which are 1 mm. long in *G. quadrivulnera* and 1.5 mm. or more in *G. viminea*, and by the capsules, *G. quadrivulnera* having the larger.

The fact that the capsules often appear square in cross-section when pressed and dried has led to some confusion as to ribbing. All capsules are terete and 8-ribbed when growing. The plant from "road to Napa Soda Springs," Jepson in 1893 (C), which JEPSON called *G. albescens* var. *micropetala* in his flora of Western Middle California, and *G. goddardii* in his revision, appears to be a rather average plant of *G. quadrivulnera* with large capsules, which have not been flattened by pressing.

In studying some 500 sheets of material of this species, I was impressed with the extreme variation in nearly all characters, and firmly believe that it is inadvisable to try to split the group into numerous ill defined segregates.

2. GODETIA PURPUREA (Curt.) G. Don in Sweet Hort. Brit., ed. III, 237. 1839.

Erect, simple, or with branches and branchlets closely crowded at summit so that a very compact inflorescence is generally formed, the flowers and capsules usually being partially concealed by the leaves; stems 1-6.5 dm. tall, finely pubescent to almost glabrous; leaves 1.5-4 cm. long, 0.3-1.8 cm. wide, lanceolate to broadly elliptic, obtuse to acuminate, short petioled; buds erect, 0.6-1.5 cm. long; hypanthium 3-7 mm. long, inner ring of hairs about one-third way from base; calyx lobes 0.4-1 cm. long, usually distinct and reflexed but occasionally partly or entirely united in anthesis, the tips often slightly free in bud; petals crimson to purple, sometimes with darker spot in center or at apex, cuneate to obovate, 0.5-2 cm. long, 0.5-1.5 cm. wide, not clawed, apex rounded, somewhat erose; filaments quite flat, dilated at base, 1-6 mm. long, unequal, the short set not over half as long as long ones; anthers subequal, 2-4 mm. long, yellow; style extending to tips of short stamens, frequently equaling long ones; stigma lobes about 1 mm. long and as broad, yellow or more commonly purple; capsule 1-3 cm. long, 3-5 mm. thick, usually conspicuously enlarged at center, strongly 8-ribbed, sessile or very short-pedicellate, not beaked, terete when growing, evidently quad-

rilateral in much of the dried material, varying in pubescence from glabrous to densely white- or yellow-lanate; seeds about 1 mm. long, slightly thicker, cresting minute.

#### Key to varieties

Leaves 1-1.8 cm. wide; capsule glabrate to pubescent; leaves seemingly glaucous.....2a. *G. purpurea* var. *typica*

Leaves 0.3-1.2 cm. wide; capsule pubescent to lanate; leaves not glaucous.....2b. *G. purpurea* var. *parviflora*

2a. *GODETIA PURPUREA* var. *typica* nom. nov.—*Oenothera purpurea* Curt. Bot. Mag. 10:t. 352. 1796; T. and G. Fl. N. Amer. 1:504. 1840, in part; S. Wats., Proc. Am. Acad. 8:596. 1873, in part; *Godetia purpurea* G. Don loc. cit.; Jepson, Univ. Cal. Pub. Bot. 2:344. 1907, in part; *Clarkia purpurea* Nels. and Macbr., Bot. Gaz. 65:64. 1918, in part; *G. willdenowiana* Spach Hist. Veg. Phan. 4:388. 1835; *G. lepida* var. *arnottii* Jepson Fl. W. Mid. Cal. 335. 1901; *G. arnottii* Jepson, Univ. Cal. Pub. Bot. 2:346. 1907; *Clarkia arnottii* Nels. and Macbr., Bot. Gaz. 65:64. 1918, in part; *Oe. prismatica* var. *dasycarpa* Leveille Monog. Onoth. 265. 1908, in small part.

Leaves broadly elliptic, 2-4 cm. long, 1-1.8 cm. wide, puberulent, yet appearing glaucous, the tips curved downward; capsule glabrate to pubescent.

Type locality, "Western Coast of North America."

Material examined, CALIFORNIA: Without locality, Douglas (G), Brewer 690 (C,G), 1842, sub. nom. *Oe. willdenowii*, authentic material *G. willdenowiana* (G); Solano Co., Elmira, K. Brandegee 121 (C,G,P,S), K. Brandegee 122 (C,G,P,S), Baker 2902 (G,P); Hartleys, Baker 2884 (C,G,P); Little Oak, Jepson in 1890, cited as typical material of *G. arnottii* (C); Marin Co., Tomales Point, Newell in 1905 (C); Sausalito, Michener and Bioletti 178 (G); Sacramento Co., Lincoln, Hall 10291 (C).

This variety is characterized by wide, seemingly glabrous leaves, which are curved downward at the tip, the capsule being glabrate to pubescent. *G. arnottii* of Jepson I place here; the two specimens he cites, from Little Oak, Jepson, and Elmira, Baker, resemble the illustration in the Botanical Magazine very closely. The type of *Oenothera arnottii* of TORREY and GRAY is entirely different and does not fit the description of JEPSON's *Godetia arnottii*. I should judge from

the discussion of *Clarkia arnottii* by NELSON and MACBRIDE, *loc. cit.*, that they have JEPSON'S *G. arnottii* in mind, as they speak of the Brandegee collection's having both glabrous and puberulent capsules (collection from Elmira, Solano Co., Calif.). The two specimens they cite, however, do not belong there. The first, Howell 703, is *G. viminea* var. *typica*, and the other, Sheldon 10975, is *G. purpurea* var. *parviflora*.

2b. GODETIA PURPUREA var. *parviflora* (S. Wats.) comb. nov.—*Oenothera lepida* var. *parviflora* S. Wats., Proc. Am. Acad. 8:597. 1873; *Oe. purpurea* T. and G. Fl. N. Amer. 1:504. 1840, in part; S. Wats., *loc. cit.* 596, in part; *Godetia purpurea* Jepson, Univ. Cal. Pub. Bot. 2:344. 1907, in part; *G. purpurea* var. *elmeri* Jepson, *loc. cit.* 345; *G. purpurea* var. *procera* Jepson, *loc. cit.* 346; *G. purpurea* var. *lacunosa* Jepson, *loc. cit.*; *G. purpurea* var. *lucunarum* Jepson Man. Fl. Pl. Cal. 679. 1925; *Clarkia purpurea* Nels. and Macbr., Bot. Gaz. 65:64. 1918, in part; *Oe. decumbens* Dougl., Curt. Bot. Mag. 56:t. 2889. 1829; Bot. Reg. 15:t. 1221. 1829; T. and G. Fl. N. Amer. 1:504. 1840; *G. decumbens* Spach Hist. Veg. Phan. 4:388. 1835; *Clarkia decumbens* Nels. and Macbr., *loc. cit.*; *Godetia lepida* Lindl., Bot. Reg. 22:t. 1849. 1836; Jepson Fl. W. Mid. Cal. 335. 1901; *Oe. lepida* H. and A. Bot. Beech. Voy. 342. 1840; T. and G. *loc. cit.*; S. Wats., *loc. cit.* 597; *Oe. lepida* var. *arnottii* S. Wats., *loc. cit.*, in part; *G. albescens* Lindl., Bot. Reg. 27: misc. 61. 1841; Jepson Fl. W. Mid. Cal. 334. 1901; *Oe. albescens* S. Wats., *loc. cit.*; *G. micropetala* Greene, Pittonia 1:32. 1887; *G. goddardii* f. *capitata* Jepson, Univ. Cal. Pub. Bot. 2:342. 1907; *G. goddardii* var. *capitata* Jepson Man. Fl. Pl. Cal. 678. 1925; *G. lanata* Elmer, Bot. Gaz. 41: 317. 1906; *Oe. prismatica* var. *dasycarpa* Leveille Monog. Onoth. 265. 1908, in part; *Oe. pulcherrima* var. *brauntoni* Leveille *loc. cit.* 261, acc. to citation; *Clarkia arnottii* Nels. and Macbr., *loc. cit.*, in part, according to citation.

Leaves 0.3–1.2 cm. wide, lanceolate to spatulate, pubescent, never appearing glaucous, tips erect; capsule pubescent to densely lanate.

Type locality, Northern California.

Representative material, OREGON: Salem, Nelson 233 (S); Turner, Nelson 755 (S); CALIFORNIA: Humboldt Co., valley of Van Duzen R., Tracy 4244 (C); Butte Co., 8 mi. N. of Oroville, Heller 11384 (C,G,S); Clear Creek, Brown

212 (C,P,S); Berry Canyon, *Brown and Heller* 5472 (G,P,S); Sacramento Co., Sacramento, *Shockley* 404 (G); Sonoma Co., Santa Rosa, *Heller* 5646 (G); Marin Co., Tiburon, *Walker* 1736 (C); Sausalito Hills, *Sulliffe* in 1927 (P); San Francisco, near Twin Peaks, *K. Brandegee* in 1908 (P); San Mateo Co., *Dutton* in 1900 (S); Alameda Co., Oakland Hills, *Bolander* 410 (C,G); Berkeley, *Bioletti* in 1891, type of *G. purpurea* var. *procera* (C); Contra Costa Co., near Martinez, *Greene* in 1889, labeled *G. micropetala* (S); Mt. Diablo, *Greene* in 1886, probably type material of *G. micropetala* (C); San Joaquin Co., near Oakdale, *Jepson* in 1896, type material of *G. purpurea* var. *lacunosa* (G,Jep); Mariposa Co., Lewis, *Congdon* in 1895 (G); Santa Clara Co., Los Gatos, *K. Brandegee* in 1905 (C); Santa Cruz Co., Santa Cruz, *Jones* in 1881 (P); Monterey Co., Lucia, *K. Brandegee* in 1909 (C); Tassajara Road, *Abrams* 5632 (P,S); San Luis Obispo Co., Santa Margarita Valley, *Mrs. Summers* in 1882 (C); Santa Barbara Co., Santa Barbara, *Elmer* 3791 (C,G,S), *Elmer* 3792, probably type material of *G. purpurea* var. *elmeri*, cited by *Jepson* as *Elmer* 3972 from Santa Barbara (C,G,S); Buellton, *Munz* 10299 (P); Ventura Co., Sulphur Mt., *Abrams* 4 (G, S); Los Angeles Co., hills N. of Los Angeles, *Lyon* 73 (G); Soldier's Home, Sawtelle, *Hasse* in 1890 (S); Elysian Park, *Braunton* in 1902 (S); San Diego Co., Cuyamaca Lake, *K. Brandegee* in 1906 (C), *Abrams* 3691 (G, S). The following collections deserve special mention because of the unusually lanate capsules: San Luis Obispo Co., Paso Robles, *Cobb* 10 (C); Arroyo Grande, *Summers* in 1886 (C); Monterey Co., between Castroville and Monterey, *K. Brandegee* in 1908 (C); Gigling, *Elmer* 4376, type collection of *G. lanata* (C,P,S); Fresno Co., Centreville, *Jepson* 2745, type of *G. goddardii* var. *capitata* (Jep).

In my opinion these forms show too much variation to be given even varietal rank. The type collection of *G. lanata* has some plants with densely lanate capsules and others that are sparsely so. The plant from Centreville, Fresno Co. (*G. goddardii* var. *capitata*), is practically identical with the type of *G. lanata*. The capsules are lanate and 2-3 cm. long; the inflorescence is practically restricted to the ends of the branchlets, and the flowers are nearly hidden by the leaves. It certainly belongs to the *G. purpurea* group. In fact, with its strongly ribbed capsules, flowers in dense clusters, rather short petals, leafy stems, and hairy ovaries, it would run directly to *G. purpurea* in *JEPSON'S* own key.

As stated, this variety is a heterogeneous group, which has been split up into numerous species and varieties, but after careful study it is considered advisable to treat it as a unit, rather than several poorly defined varieties, which it would be impossible to distinguish.

Some of the forms, such as a series from Elizabeth Lake, Los Angeles Co., *Hoffmann* in 1927 (P,SB), show every gradation from plants which are very evidently *G. purpurea* to those which resemble *G. quadrivulnera* very closely. In fact, it is frequently very difficult to distinguish *G. purpurea* var. *parviflora* from depauperate specimens of *G. quadrivulnera*.

3. GODETIA AMOENA (Lehm.) G. Don in Sweet Hort. Brit. ed. III, 237. 1839.

Erect, simple to diffusely branching; stems 1.5–10 dm. tall; leaf blades narrowly to broadly lanceolate, 2–6 cm. long, 0.2–1 cm. wide, acute to blunt, tapering to a petiole 0.5–1.5 cm. long; buds erect, or sometimes drooping slightly, 1–4 cm. long; hypanthium 4–10 mm. long, proportionately shorter in small-flowered forms, green or yellow on outside, white to greenish pink on inside, inner ring of hairs one-third to one-half way from base; calyx lobes 0.8–2.5 cm. long, generally united in anthesis, less commonly reflexed in pairs, or even all distinct; petals pale pink to purple, often with darker purple spot in center, or with dark purple base, cuneate to obovate, 1.5–4 cm. long, 1–3 cm. wide, tapering uniformly to base, or occasionally abruptly narrowed, forming a claw as much as 1.5 mm. long, apex rounded, truncate, or retuse, usually somewhat erose; filaments flattened, 2–12 mm. long, short set from one-half as long to nearly as long as long ones; anthers subequal, 5–12 mm. long, yellow or more commonly purple with yellow tips, tips usually sterile, but whole anther often either fertile or sterile; style varying in length from less than that of short stamens to nearly equal to long ones; stigma lobes varying from 1.5 mm. long to the more common type 3–7 mm. long, about 0.5 mm. wide, yellow; capsules 1.5–5 cm. long, 1.5–4 mm. thick, linear, or enlarged somewhat at or above the center, beakless, or with beak as much as 1 cm. long, sessile or with pedicel as much as 3 cm. long, at first (i.e., when immature) deeply 4-sulcate and with rather inconspicuous nerve between the grooves, at maturity and in dried material becoming terete with the intersulcate nerves showing plainly; seeds 0.5 mm. long, 1.5 mm. thick, brown, cresting not prominent.

## Key to varieties

- A. Stigma lobes linear, 2-7 mm. long.
  - B. Capsule enlarged above center; petals usually with large purple spot in center. Sonoma County, California
    - 3a. *G. amoena* var. *sonomensis*
  - B. Capsule not enlarged above center; petals with or without purple spot.
    - C. Capsule 1.5-2 mm. thick, 4-5 cm. long, nearly sessile; petals never with spot. Butte County, California
      - 3b. *G. amoena* var. *albicaulis*
    - C. Capsule usually over 2 mm. thick, less than 4 cm. long, usually pedicelled; petals with or without blotch. From Santa Cruz, California, to British Columbia
      - 3c. *G. amoena* var. *typica*
  - A. Stigma lobes ovoid, not over 2 mm. long.
    - B. Capsule blunt or short-beaked. Plants of Oregon and Washington. . . . . 3d. *G. amoena* var. *gracilis*
    - B. Capsule conspicuously beaked. Plants of Central California
      - 3e. *G. amoena* var. *concolor*

3a. *GODETIA AMOENA* var. *sonomensis* var. nov.—Capsule sessile, considerably enlarged a little above the middle, blunt; petals 1.5-4 cm. long, dark lavender, usually with carmine or purple spot in center; stigma lobes 3-7 mm. long, linear. (Capsulae supra medio dilatatae, obtusae, sessiles; petalis maculatis.)

Type,  $\frac{1}{2}$  mi. E. of Glen Ellen, Sonoma Co., California, June 7, 1928, C. L. Hitchcock 18 (Pomona College Herb. no. 153238).

Other material examined, CALIFORNIA: Sonoma Co., Burke's Sanitarium, Heller 5752 (G,S); Glen Ellen, Michener and Bioletti in 1893 (C,G,P), Michener and Bioletti 682 (C); 3 mi. S.E. of Glen Ellen, C. L. Hitchcock 19 (P); Santa Rosa, Heller 5682 (G,P,S);  $5\frac{1}{2}$  mi. S. of Santa Rosa, C. L. Hitchcock 21 (P); near Healdsburg, King in 1897 (C); between Cloverdale and Geyserville, M. S. Baker 670 (C).

This variety is very showy. The petals usually have a crimson spot in the center but this is not always so. Capsules are long and decidedly enlarged above the middle. It is restricted to Sonoma Co., California.



3b. *GODETIA AMOENA* var. *ALBICAULIS* Jepson, Univ. Cal. Pub. Bot. 2:329. 1907.

Capsule sessile or nearly so, 4–5 cm. long, 1.5–2 mm. thick, linear, beak 5–10 mm. long; petals 2–4 cm. long, light lavender, without spot; stigma lobes 3–6 mm. long, linear.

Type locality, Rancho Chico, Butte Co., California.

Material examined, CALIFORNIA: Butte Co., *Mrs. Austin* 34 (C,P), *Fremont* 438 (G); Shasta Co., *Pitt River*, *Smith* 315 (G); Butte Co., *Lemmon's Ranch*, *Lemmon* 4546 (G); Chico, *Mrs. Bidwell* in 1879 (G); Little Chico, *Mrs. Bruce* 1954 (P,S); banks of Little Chico Creek, July, 1896, 848 (C); De Salba, *Edwards* in 1917 (P,S).

3c. *GODETIA AMOENA* var. *typica*, nom. nov.—*Oenothera amoena* Lehm., Ind. Sem. Hort. Hamb. 8. 1821; *Pugill.* Pl. 1:811. 1828; T. and G. Fl. N. Am. 1:503. 1840; S. Wats., Proc. Am. Acad. 8:599. 1873; *Godetia amoena* G. Don *loc. cit.*; *Lilja* in *Linnaea* 15:265. 1841; Jepson, Univ. Cal. Pub. Bot. 2:325. 1907; *Clarkia amoena* Nels. and Macbr., Bot. Gaz. 65:62. 1918; *G. amoena* f. *pygmaea* Jepson, *loc. cit.* 328; *G. amoena* var. *pygmaea* Jepson Man. Fl. Pl. Cal. 677. 1925; *G. amoena* f. *huntiana* Jepson, Univ. Cal. Pub. Bot. 2:329. 1907; *G. amoena* var. *huntiana* Jepson Man. Fl. Pl. Cal. 677. 1925; *G. amoena* var. *lindleyi* Jepson, Univ. Cal. Pub. Bot. 2:329. 1907; *Clarkia amoena* f. *lindleyi* Nels. and Macbr., *loc. cit.*; *Oenothera prismatica* var. *amoena* Lev. Monog. Onoth. 266. 1908, in part; *Oe. roseoalba* Bernh., Ind. Sem. Hort. Erford (1824), *ex Reichb.* Ic. Bot. Exot. 1:34, pl. 47. 1827; Sweet Brit. Fl. Gard. t. 268. 1828; *Oe. lindleyi* Dougl., Bot. Mag. 55:t. 2832. 1828; T. and G. *loc. cit.*, 503; *Godetia lehmanniana* Spach Hist. Veg. Phan. 4:391. 1835, *Nouv. Ann. Mus. Par. III*, 4:392. 1835; *G. lindleyana* Spach Hist. Veg. Phan. 4:392. 1835, *Nouv. Ann. Mus. Par. III*, 4:392. 1835; *Oe. lindleyana* Steud. Nom. ed. II, 2:207. 1841; *G. rubicunda* Lindl., Bot. Reg. 11:t. 1856. 1836; *Oe. rubicunda* H. and A. Bot. Beech. Voy. 342. 1840; T. and G. *loc. cit.* 502; *G. vinosa* Lindl., Bot. Reg. 22:t. 1880. 1836; *Oe. vinosa* T. and G. *loc. cit.* 503; *Oe. macrantha* Nutt. in H. and A. Bot. Beech. Voy. 342. 1840; *G. macrantha* Lilja, Tidnung (1840), *ex cod.* in *Linnaea* 15:265. 1841; *G. grandiflora* Lindl., Bot. Reg. 27: misc. p. 61. 1841; Jepson, Univ. Cal. Pub. Bot. 2:347. 1907, in part; *Oe. grandiflora* Wats., Proc. Am. Acad. 8:596. 1873, in part; *G. cau-*

*rina* Abrams ex Piper, Contr. U. S. Nat. Herb. 11:410. 1906; *Clarkia caurina* Nels. and Macbr., Bot. Gaz. 65:62. 1918; *G. blasdalei* Jepson, loc. cit. 330; *Clarkia superba* Nels. and Macbr., loc. cit. 60.

Capsule usually pedicelled, not noticeably enlarged above center, blunt or short beaked; petals 1-4 cm. long, light pink to purple, with or without central blotch or base of purple; stigma lobes 2-7 mm. long, linear.

Type locality, "America septentrionalis."

Representative material, without locality: "*e* Lehmann *ipse*," authentic material of *O. amoena* (G); "*Godetia vinosa* Lindl. Spach M. Par.," authentic material of *G. vinosa* (G); "*Spach*, M. Paris 1838," authentic material of *G. rubicunda* (G); "*Oe. lindleyi*, cult., Sartwell *e. Sem. Hort. Soc. Lond.* 1833" (G); BRITISH COLUMBIA: Victoria, Macoun 283 (G); WASHINGTON: between Olympia and Gate City, A. A. and E. G. Heller 4051 (C,G,P,S); Olympic Mts., Elmer 2565, type collection of *G. caurina* (S); Chenoweth, Suksdorf 2129 (C,G); OREGON: without locality, Nuttall 1866, type of *Oe. macrantha* (G); Willamette R., below Portland, Sheldon 10864 (G,P,S); Eugene, Nelson 330 (S); CALIFORNIA: Siskiyou Co., Klamath R., Butler 747 (S); Del Norte Co., Crescent City, Kildale 1035 (S); Humboldt Co., Shelter Cove, Tracy 4998 (C); Garberville, Abrams 8229 (P,S); Trinity Co., Duzen R., Abrams 6020 (S); Shasta Co., Mt. Shasta, Hall and Babcock 4088, small-flowered form (C); Plumas Co., Mrs. Ames 1875 (G); Mendocino Co., Comptche, Walker 385 (C); Lake Co., Blue Lakes, July 16, 1897, Jepson 13336, type *G. amoena* f. *huntiana* (Jep); Saratoga Springs, Jepson 13338 (Jep); Colusa Co., 3 mi. W. of Leesville, Heller 12354 (G,S); Napa Co., Calistoga, K. Brandegee 120 (C,G,P,S); Conn Valley, May 1, 1894, Jepson 13339, type of *G. amoena* f. *pygmaea* (Jep); Sonoma Co., Glen Ellen, K. Brandegee in 1908 (C,P); Marin Co., Olema, Elmer 4436 (C,P,S); Tiburon Peninsula, Heller 5726 (G,P,S); Alameda Co., Oakland Hills, Bolander in 1865 (C,G); Cedar Mt., Elmer 4436 (C,P,S); Contra Costa Co., 2 mi. S. of Dublin, C. L. Hitchcock 16 (P); San Francisco, between Baker's Beach and Land's End, K. Brandegee in 1906 and 1907 (C); San Mateo Co., near Crystal Springs Lake, Elmer 4310 (C,P,S); Milbrae, Eastwood 331 (G); Santa Cruz Co., Watsonville, Elmer 5053 (C,P,S); grade above Santa Cruz, C. L. Hitchcock 12 (P); Santa Clara Co., Mission Peak, San Jose, Brewer in 1864 (G); Saratoga, Pendleton 395 (C); foothills W. of Los Gatos, Heller 7479 (C,G,S); NEVADA: Ormsby Co., Ash Canyon, Baker 980, small-flowered (P); Carson City, Jones in 1897, small-flowered (P).

Excepting *G. quadrivulnera*, *G. amoena* has the greatest distributional range of any species of *Godetia*, and is one of the most variable. Several species have been described from color variants, among them *G. vinosa*, *G. rubicunda*, *G. roseo-alba*, and *G. lindleyi*. Coloration is so

variable here that I am unable to recognize these even as varieties, although there does seem to be some tendency for the northern forms to have spotted petals, but much of the material from the San Francisco Bay region also shows this coloration. The length of capsule, length of pedicel, size and coloration of petals, beaking of capsule, and even the length of stigma lobes all vary greatly.

*G. caurina* was described from a plant with glabrous anthers, but is identical with *G. amoena* in every other respect. Due to the fact that the pubescence of the anthers is so scattered that it is difficult to distinguish, and because other plants examined from the same locality do have a few scattered hairs on the anthers, their mere presence or absence does not seem to merit nomenclatural recognition.

*G. amoena* var. *pygmaea* was described from a depauperate specimen. In its subsessile capsule, which is slightly thickened upward, it suggests the var. *sonomensis* and is intermediate between that variety and var. *typica*. *Huntiana* is also considered unworthy of recognition. The type from Blue Lakes is not an unusual form. The "3-forked blotch" is not uncommon; for example, a specimen from 20 mi. N. of Laytonville, Mendocino Co., *Munz* 9884 (P), has petals with a three-forked blotch in the center but is typical material in every respect. The petals of *Jepson's* plant are 1.8 cm. long, which is near the average. The other specimen cited by JEPSON in his original description, from Saratoga Springs, appears to have had the top eaten off, so that it is not strange if the resulting growth was somewhat unusual.

There are several local variants worthy of mention. *K. Brandege* made several collections from McDowell Avenue, San Francisco, that have truncate petals, the base red, central portion yellow and apex lavender. Several collections from the north side of San Francisco Bay, among them: Pt. Isabel, *Blasdale* in 1893, type of *G. blasdalei* (C), *K. Brandege* in 1908 (C,P);  $\frac{1}{2}$  mi. N. of Tiburon, *C. L. Hitchcock* 25 (P), have rather thick capsules, and petals that are often retuse, although all the flowers on one plant are not alike in this respect. Plants of high altitudes usually have small flowers with all the parts reduced.

*Godetia amoena* can usually be distinguished from *G. bottae* by the longer hypanthium and stigma lobes, but occasionally individuals

are found that have as short hypanthia as the latter species. In the field they can readily be told by the difference in the immature capsule, that in *G. amoena* being 4-sulcate and that in *G. bottae* being terete and faintly nerved. With dried material, the only way to distinguish between the two species is by the position of the inner ring of hairs on the hypanthium. In *G. amoena* this ring is found at a point below the middle, in *G. bottae* it is above the middle.

3d. *GODETIA AMOENA* var. *gracilis* (Piper) comb. nov.—*Godetia gracilis* Piper in Piper and Beattie Fl. N. W. Coast 251. 1915; *Clarkia gracilis* Nels. and Macbr., Bot. Gaz. 65:63. 1918.

Capsule nearly sessile, blunt or short beaked; petals 1-2 cm. long, stigma lobes oval, about 1 mm. long.

Type locality, Silvertown, Oregon.

Representative material, BRITISH COLUMBIA: near Victoria, Fletcher 811a (G); WASHINGTON: Klickitat Co., Suksdorf 23 (G); Bingen, Suksdorf 5606 (G); Walla Walla region, Tweedy in 1883 (S); Vancouver Island, Macoun 42 (G), Rosendahl 1849 (G); OREGON: Josephine Co., Takilma, Peck 7928 (G); Waldo, Abrams and Benson 10366 (S); Clakamas Co., Willamette Falls, Sheldon 12216 (S); Crook Co., Grizzly Butte, Leiber (G,P,S); Hood River, Henderson in 1882 (S); summit of Parrott Mt., Nelson 2719 (G); Oregon, Elihu Hall 192, type collection of *G. gracilis* (G). The following are intermediate between var. *gracilis* and var. *typica*: Friday Harbor, San Juan Islands, Washington, Dr. and Mrs. Zeller 897 (G); Hood River Co., Henderson 834 (G).

This variety is characterized by the rather small flowers, very short beaked capsule and short stigma lobes. Since it has the characteristic terete, nerved capsule of *G. amoena* when dried, it is not deemed deserving of specific rank.

3e. *GODETIA AMOENA* var. *concolor* Jepson Fl. W. Mid. Cal. 334. 1901.—*Clarkia amoena* f. *concolor* Nels. and Macbr., Bot. Gaz. 65:62. 1918.

Capsule pedicelled, beak 3-7 mm. long; petals 1-1.5 cm. long; stigma lobes oval, about 1 mm. long.

Type locality, Pope Valley Grade, Napa Co., California.

Material seen, Pope Valley Grade, Jepson 13337, type (Jep); K. Brandegee in 1909 (C,P,S); El Dorado Co., New York Ravine, K. Brandegee in 1907 (C,P); Butte Co., Mrs. Bruce 2419 (P); Kelsey, Jones in 1883 (P). The following collection approaches this variety: Yolo Co., Cache Creek Canyon, Baker 2891 in part (G,P).

This variety is easily confused with small-flowered forms of *G. amoena* var. *typica*. The stigma lobes, as in var. *gracilis*, are much shorter than in the typical form. It resembles var. *gracilis* very closely but has a well beaked capsule, while the capsule in the latter variety is not beaked.

4. *GODETIA WHITNEYI* (Gray) T. Moore Flor. and Pomol. 101. 1871.—*Oenothera whitneyi* Gray, Proc. Am. Acad. 7:340. 1865; *Clarkia whitneyi* Nels. and Macbr., Bot. Gaz. 65:61. 1918; *Oe. auricula* var. *whitneyi* Leveille Monog. Onoth. 270. 1908; *Oe. grandiflora* Wats., Proc. Am. Acad. 8:596. 1873, in part; *Godetia grandiflora* Jepson, Univ. Cal. Pub. Bot. 2:347. 1907, in part.

Erect, stout, simple or with closely crowded branches above; stems 2-4.5 dm. tall, finely pubescent; leaf blades lance-ovate to ovate, 3.5-6 cm. long, 0.8-1.5 cm. wide, acute to acuminate, petiole 2-10 mm. long; inflorescence finely pubescent; buds erect, 3-4 cm. long, acuminate; hypanthium broad at top, 6-9 mm. broad in pressed specimens, 8-11 mm. long, ring of hairs within about one-third way from base; calyx lobes 1.5-3 cm. long, united in anthesis, tips not free in bud; petals lavender with a splotch of deep red or purple toward base, cuneate to obovate, 4-6 cm. long, 2.5-4 cm. wide, rounded to retuse at apex, rather abruptly narrowed at base to form a 1.5-2 mm. claw; filaments unequal, 3-9 mm. long, strap-shaped, dilated at base; anthers subequal, 9-15 mm. long, purple, tinged with yellow above, fertile to tip; style 2.5-3 cm. long, exceeding stamens, extending half the length of petals; stigma lobes linear, yellow, 6-7 mm. long; ovary densely canescent; capsule broadly fusiform, 1.5-2.5 cm. long, 0.5-0.7 cm. thick, round in cross-section, with 8 prominent ribs, sessile or with pedicel as much as 3 mm. long; seeds 1.5 mm. long and half as thick, brown, covered with minute cellular pubescence, creasing fairly pronounced.

Type locality, Shelter Cove, Humboldt Co., California.

Material seen, Shelter Cove, Bolander 6534, type collection of *Oe. whitneyi* (C,G); Peirson in 1927 (P); California, Vasey in 1876 (G); from Thomson's seeds in 1871 (G). There are also several sheets from Burbank's garden (C), which are evidently of this species.

WATSON combines this species with *Oe. grandiflora*, and so does JEPSON. But as NELSON and MACBRIDE, Bot. Gaz. 65:60. 1918,

point out, the description of *Oe. grandiflora* says "fructu lineari 4-sulcato tereti pubescente," a character which proves the relationship of that species to *G. amoena*, the only species of *Godetia* with a 4-sulcate capsule. *G. whitneyi* certainly does not have either a 4-sulcate or a linear capsule, and, as GRAY pointed out in describing it, it is related to *G. purpurea* in habit and capsule character; on the other hand, it is similar to *G. amoena* in hypanthium, stigma lobes, and filaments.

5. *GODETIA VIMINEA* (Dougl.) Spach Hist. Veg. Phan. 4:388. 1835.

Erect, branching from middle, more rarely from base, frequently simple; stems 1.5-10 dm. tall, finely white-pubescent; flowering branches short or as much as 40 cm. long; leaf blades lance-oblong to spatulate, 2-5 cm. long, 0.2-0.6 cm. wide, acute to rounded at apex, short petioled; buds erect, 1.5-2.5 cm. long and one-third as thick, tapering very abruptly at apex to a point 0.5 mm. long; hypanthium 0.6-1.2 cm. long, slender, green or yellow, tinged with lavender, inner ring of hairs about one-third way from base; calyx lobes 0.7-1.4 cm. long, greenish-yellow, reflexed in pairs, or more commonly all distinct in anthesis, the tips usually slightly free in bud, very finely pubescent to rather densely pilose; petals cream-yellow at base, with large central purple spot, crimson with purple spots, or plain lavender or purple 1.3-2.5 cm. long, 1-2.2 cm. wide, cuneate, without claw, the apex rounded and usually somewhat erose; filaments strap-shaped, dilated at base, 2-6 mm. long, unequal, the long set about three times as long as others; anthers subequal, 5-7 mm. long, white or yellow or more rarely lavender; style equaling or slightly exceeding longer stamens; stigma lobes elliptic, about 1.5 mm. long and one-third as broad, yellow to deep purple; young ovaries very short, approximately one-third length of bud, pubescent to pilose; capsule 1-3 cm. long, 2-4 mm. thick, plainly 8-ribbed, in pressed specimens often appearing quadrilateral, sessile or with pedicel 2 mm. long, with beak about 1 mm. long; seeds 0.75 mm. long, slightly broader, smooth, cresting inconspicuous.

#### Key to varieties

- A. Capsules usually somewhat enlarged at center; petals not spotted  
5a. *G. viminea* var. *typica*

A. Capsules linear, or at least not enlarged at center; petals usually spotted.

B. Petals yellow toward base.....5b. *G. viminea* var. *williamsonii*

B. Petals crimson.....5c. *G. viminea* var. *incerta*

5a. GODETIA VIMINEA var. **typica**, nom. nov.—*Oenothera viminea* Dougl. ex Hook., Bot. Mag. 55:t. 2873. 1828; T. and G. Fl. N. Am. 1:503. 1840, in part; S. Wats., Proc. Am. Acad. 8:598. 1873; *Gode-tia viminea* Spach, loc. cit.; Jepson, Univ. Cal. Pub. Bot. 2:336. 1907, in part; *Clarkia viminea* Nels. and Macbr., Bot. Gaz. 65:64. 1918, in part; *Oe. arnottii* T. and G. loc. cit.; *G. arnottii* Walp. Rep. 2:88. 1843; *C. arnottii* Nels. and Macbr., loc. cit., in part, from citation; *Oe. lepida* var. *arnottii* S. Wats., loc. cit., 597, in part; *Oe. auricula* var. *tenella* subvar. *williamsonii* f. *vimineiformis* Leveille Monog. Onoth. 272. 1908.

Petals lavender; anthers yellow; capsule enlarged at center, 3-4 mm. thick; flowers borne in compact spikes; hypanthium 6-9 mm. long.

Type locality, interior of northern California.

Representative material, OREGON: Coast ranges, *Howell* and *Henderson* in 1882 (G); Multnomah Co., *Howell* 138 (G); Eugene, *Bradshaw* 1749 (S); Monroe, *Munz* 9900 (P,S); Grant's Pass, *Henderson* 356 (C,S); the Dalles, *C. Davidson* (P); CALIFORNIA: El Dorado Co., Blue Ravine, *K. Brandegee* in 1907 (C); Sweetwater Creek, *K. Brandegee* in 1908 (C); Mendocino Co., Leggett Valley, *Munz* 9883 (P,S); Humboldt Co., near Garberville, *Munz* 9887 (P); Humboldt Co., Dinsmore's Ranch, *Tracy* 3957 (G); Marin Co., Olema, *K. Brandegee* in 1908 (P), *Walker* 1199 (C); Oakland Hills, *Bolander* 282 (G); Stanislaus Co., Knight's Ferry, *Dr. Bigelow* (G); Ventura Co., Sulfur Mts., *Abrams* and *MacGregor* in 1908, capsules unusually pilose (G,P,S); California, *Douglas*, type of *Oe. arnottii* (G); foothills W. of Los Gatos, *Heller* 7446 (G,S).

JEPSON, Univ. Cal. Pub. Bot. 2:336. 1907, calls attention to the fact that *G. viminea* and *G. williamsonii* are very closely related and he treats the latter as a synonym of the former. I find, however, that the material from Oregon and from central coastal and northern California has a larger, thicker capsule than the material from the Sierra Nevadas, and the flowers in the material from the former localities are less inclined to be spotted. The plate in the Botanical Magazine shows an unspotted flower, and a later plate of *Oe. viminea*, Bot. Reg. 15:1220. 1829, represents a plant with large,

thick capsules and faintly spotted petals. Because of the fact that there seems to be this difference between the Sierra Nevadan plants and the others, the groups are kept as varieties. The type of *Oe. arnottii* resembles *G. viminea* var. *typica* in size and coloration of petals, and in type of inflorescence. It certainly is not in any way like *G. arnottii* Jepson, which is "wholly glabrous; calyx tube 3 mm. long; petals 1.3 cm. long," as the *Douglas* specimen is sparingly pubescent, and has a 6 mm. calyx tube, and petals 1.6 cm. long.

5b. *ODETIA VIMINEA* var. *congdonii* Jepson Univ. Cal. Pub. Bot. 2:338. 1907. *Godetia williamsonii* Dur. and Hilg., Pac. R. R. Rep. 5<sup>3</sup>:7. 1855; *Oenothera williamsonii* S. Wats., Proc. Am. Acad. 8:597. 1873; *Oe. auricula* var. *tenella* subvar. *williamsonii* Leveille Monog. Onoth. 271. 1908; *Oe. viminea* T. and G. Fl. N. Am. 1:503. 1840, in part; *G. viminea* Jepson, Univ. Cal. Pub. Bot. 2:336. 1907, in large part; *Clarkia viminea* Nels. and Macbr., Bot. Gaz. 65:64. 1918, in part; *Oe. prismatica* var. *viminea* Leveille loc. cit. 265.

Petals yellow to lavender with purple spot in center; anthers yellow; capsule not enlarged at center, usually not over 2 mm. thick; flowers scattered on long flowering branches; hypanthium 0.8–1.2 cm. long.

Type locality, Hetch-Hetchy Valley, Tuolumne Co., California.

Representative material, CALIFORNIA: without locality, *Bridges* 107 (G); *Hartweg* 1727 (G); *Bolander* 6367 (C,G); Shasta Co., Pitt to Baird, *Eastwood* 1405 (G); Sierra Co., Sierra Valley, *Lemmon* in 1873 (G); El Dorado Co., near Fyffe, *K. Brandegee* in 1908 (G,P); Nevada Co., near Wolf Creek, *Eastwood* 3442 (G); Amador Co., 5 mi. N.E. of Jackson, *C. L. Hitchcock* 31 (P); New York Falls, *Hansen* 36 (C,P,S); Tuolumne Co., Hetch-Hetchy Valley, *Congdon* in 1896, type of *G. viminea* var. *congdonii* (C,G); Hog Ranch, *Congdon* in 1896 (G); Mariposa Co., Westfall Road, *Congdon* in 1895 (G,S); above Yosemite, *Lemmon* 246 (G); Yosemite, *Spencer* in 1917 (G); Wawona, *Abrams* 5402 (S); Madera Co., Raymond, *Mrs. Tompkins* in 1894 (S); Tulare Co., Middle Tulare R., *Purpus* 5574 (G); Fresno Co., Big Sandy Creek, *MacDonald* in 1915 (G); near Toll House, *Hall and Chandler* 15 (C); Pine Ridge, *Hall and Chandler* 203 (S); Kern Co., vicinity of Poso Creek Valley, *Dudley* 563 and 550 (S); Tejon Canyon, *Coville and Funston* 1217 (C). Several collections from Live Oaks, Mokelumne R., *Rattan* in 1885 and in 1886 (G,S), are peculiar in that the flowering spike is greatly shortened.

Variety *congdonii* shows considerable variation in the pubescence of calyx lobes and young capsules. For example, in a collection from



Hetch-Hetchy Valley, *Bioletti* in 1900 (C), the lobes are pilose; in that of *Chestnut* and *Drew* in 1889, from same locality (C), the capsule is pilose but the calyx lobes are not, and in the following collections individual plants can be found which have some flowers with pilose lobes, while others have merely pubescent ones: Tuolumne Co., Hog Ranch, *Hall* and *Babcock* 3313 (C, P); El Dorado Co., 12 mi. E. of Placerville, *K. Brandegee* in 1908 (C); Amador Co., near Jackson, *Mullikin* 115 (C,S).

5c. *GODETIA VIMINEA* var. *INCERTA* Jepson, Univ. Cal. Pub. Bot. 2:339. 1907.

Petals crimson with deeper-colored spot in center; anthers yellow or lavender; capsule not enlarged at center, about 2 mm. thick; flowers borne in long, loose spikes; hypanthium 0.8–1.2 cm. long.

Type locality, Yosemite Valley, California.

Representative material, CALIFORNIA: Yosemite Valley, *Parish* 4219 (S), *Cook* and *Reynolds* (P), *Mrs. Bacon* in 1902 (S), *Kennedy* 3022 (C), *Redfield* 1116 (C), *Hall* in 1900 (C), *Hall* 9109 (C), *Mrs. Tompkins* in 1894 (S), *S. H. B.* in 1894 (S), *Parry* (S), *M. S. Baker* 43c–25 (S); Tulare Co., Eshome Valley, *Mrs. Clemens* in 1910 (P).

6. *GODETIA PARVIFLORA* (H. and A.) Jepson, Univ. Cal. Pub. Bot. 2:339. 1907.

Erect and simple, or more commonly branching from base and ascending; stems 1–4 dm. tall, branches filiform and wiry; leaf blades linear-lanceolate to oblanceolate, 1–4 cm. long, 1.5–5 mm. broad, acuminate to rounded, short petioled; buds erect, 1.5–2.5 cm. long, acuminate; hypanthium slender to broad at summit, 0.5–1.5 cm. long, usually having a swelling at top of ovary, ring of hairs within one-fifth to one-third way from base; calyx lobes 0.5–1.5 cm. long, green to rose, united in anthesis, reflexed in pairs, or all distinct, the tips sometimes free in bud; petals varying in color from cream with crimson spot in center to cream or yellow at base and crimson above, with or without spots, or light crimson with deeper-colored spot, or petals solid crimson, triangular to obovate, 1–2.5 cm. long, 0.7–2.5 cm. wide, apex truncate to rounded, without claw; filaments unequal to subequal, 1–6 mm. long, broadly strap-shaped, dilated slightly at base; anthers subequal, 3–7 mm. long, yellow to lavender; style equalling longest anthers or nearly equalling petals; stigma lobes

oval or linear, 1.5-3 mm. long and one-third as broad, lavender to purple; capsule 1-2.5 cm. long, 0.1-0.2 cm. wide, 8-ribbed, in fresh specimens terete but in dried specimens apparently quadrilateral, pubescent, usually conspicuously curved, sessile or with pedicel as much as 2 mm. long, not beaked or with very short beak; seeds nearly equilateral, 1 mm. long, dark brown, creasing minute.

#### Key to varieties

A. Filaments subequal; style usually exceeding longer stamens; petals solid crimson, with or without purple spot toward base

6a. *G. parviflora* var. *typica*

A. Filaments unequal; style usually about equaling longer stamens; petals not solid crimson.

B. Anthers lavender; stigma lobes lavender; petals cream with purple spot.....6b. *G. parviflora* var. *luteola*

B. Anthers yellow; stigma lobes purple; petals red with yellow base.....6c. *G. parviflora* var. *margaritae*

6a. *GODETIA PARVIFLORA* var. *typica*, nom. nov.—*Godetia parviflora* Jepson, Univ. Cal. Pub. Bot. 2:339. 1907, in part; *Oenothera viminea* var. *parviflora* H. and A. Bot. Beech. Voy. 342. 1840; *Oe. tenella* Wats., Proc. Am. Acad. 8:598. 1873, in part; *G. quadrivulnera* var. *tenella* Jepson Fl. W. Mid. Cal. 334. 1901, in part.

Filaments subequal, 1-4 mm. long; anthers usually lavender; hypanthium 0.7-1.5 (usually about 1) cm. long; style exceeding longer stamens, nearly equaling petals; stigma lobes purple; petals cuneate, 1-2 cm. long, 0.8-2 cm. wide, tapering very uniformly to base; apex rounded, truncate or even retuse, crimson with central spot of purple or solid crimson; capsules 1-1.5 cm. long.

Type locality, Monterey, California.

Representative material, CALIFORNIA: San Luis Obispo Co., Cholami, Lemmon 4567 and 4587 (G); Paso Robles, Cobb 3 and 15 (C); Santa Margarita, K. Brandegee in 1911 (C); 3½ mi. E. of Templeton, Wiggins 2070 (S); 3 mi. S. of Atascadero, C. L. Hitchcock 8 (P); La Panza, Mrs. Summers in 1888, branches stouter than usual (C); Monterey Co., Jolon Valley, Hall 9974 (C); Jolon, K. Brandegee 77 and 123 (C,G,P,S); Santa Lucia Mts., K. Brandegee in 1909 (C); between Jolon and San Antonio Mission, Abrams 6487 (P,S); San Antonio Creek, above Mission, Dudley in 1895 (S); Salinas Valley, Brewer 519 (C,G); "California," Douglas, type material (G); Ventura Co. (?-probably wrong locality), Sweet in 1917 (S).

It is doubtful whether *G. parviflora* should be considered a distinct species, or a variety under *G. viminea*. The rather striking coloration, the filiform basal branching and the strongly curved capsule, however, seem to separate it clearly. If one recognizes *G. parviflora* as specific, var. *margaritae* and var. *luteola* must be considered as related forms, since they also have basal branching and curved capsules. This would mean two rather closely related species, with *G. parviflora* confined to the coast from San Luis Obispo to Santa Cruz, and *G. viminea* and varieties confined largely to the interior.

The description of *Oenothera tenella* Cavanilles, Icon. 4:66, t. 396. 1797, does not fit the species, and the plate shows a plant with very strongly angled capsules. The plate in the Botanical Magazine, 50:t. 2424. 1823, does not resemble *G. parviflora*; the coloration is different, the long hypanthium is not in evidence, and the capsules are not curved. The material from South America, labeled *G. tenella*, resembles *G. quadrivulnera* more than it does *G. parviflora*, but is not identical with either. Due to this uncertainty I consider it wise to reject the name for North American plants until the species from the Southern Hemisphere can be more thoroughly studied.

6b. GODETIA PARVIFLORA *luteola* var. nov.—Filaments unequal, 1-3 mm. long, long ones about twice the length of others; anthers yellow; hypanthium usually about 1 cm. long; style equaling longer stamens; stigmas lavender; petals cuneate-obovate, 1-2 cm. long, 0.8-1.5 cm. wide, apex rounded, cream with large central crimson spot; capsules 1-1.5 cm. long. (Antherae luteolae; stigmatibus ostrinibus; petalis ochroleucis cum magna sanguinea macula.)

Type, top of grade on road between Atascadero and Morro Beach, San Luis Obispo Co., California, June 17, 1928, C. L. Hitchcock 40 (Pomona College Herb. no. 153223).

Other material: McGinnis, 25 mi. N.E. of San Luis Obispo, Palmer 143 (C,G); Atascadero to Atascadero Beach, Abrams 7653 (S).

This peculiar form was first noticed in the Gray Herbarium material. As an opportunity came to collect in the San Luis Obispo region in the summer of 1928, I searched for these plants and found them growing in abundance in this spot (type locality).

6c. *GODETIA PARVIFLORA* var. *margaritae* (Jepson) comb. nov.—*Godetia viminea* var. *margaritae* Jepson, Univ. Cal. Pub. Bot. 2:339. 1907.

Filaments unequal, 2–6 mm. long, the longer ones two to three times as long as the others; anthers lavender; hypanthium 0.3–1.2 (usually about 0.6) mm. long; style equaling longer stamens; stigma lobes purple; petals cuneate-obovate, apex rounded, 1.2–2.5 cm. long, 1–2.5 cm. wide, base yellow, upper part wine-colored, red or dark lavender, occasionally with light red spot in center; capsules 1–2.5 cm. long.

Type locality, Santa Margarita Valley, San Luis Obispo Co., California.

Material examined, CALIFORNIA: San Luis Obispo Co., road from Arroyo Grande to Huasna, *Eastwood* 14990 (P); highway between Pismo and Arroyo Grande, *C. L. Hitchcock* 42 (P); sandhills near Pismo Beach, *Abrams* 6519 and 6520 (S); Price Canyon, *K. Brandegee* 126 (C,G,P,S), *C. L. Hitchcock* 4 and 36 (P); near San Luis Obispo, *Lemmon* 4615 (G); Santa Margarita Valley, *Mrs. Summers* in 1882, in part (C), *Mrs. Summers* 323, type of *G. viminea* var. *margaritae* (C).

Although the type of *G. viminea* var. *margaritae* is very badly faded, I believe it is identical with the material from Pismo Beach and vicinity; the large flowers, with red and yellow petals, are very conspicuous. As previously stated (*cf.* discussion under *G. parviflora* var. *typica*), this variety shows much closer affinity to *G. parviflora* than to *G. viminea*, where it was placed by JEPSON, and for this reason I am transferring it to the former species.

7. *GODETIA HISPIDULA* S. Wats., Bot. Calif. 1:231. 1876.—*Oenothera hispidula* Wats., Proc. Am. Acad. 8:599. 1873; *G. arcuata* Jepson, Univ. Cal. Pub. Bot. 2:335. 1907; *G. hansenii* Jepson, *loc. cit.* 336; *Oe. pulcherrima* Leveille Monog. Onoth. 261. 1908, in part, according to citations; *Clarkia arcuata* Nels. and Macbr., Bot. Gaz. 65:62. 1918.

Erect, simple or branching from base; stems 1–6.5 dm. tall; leaf blades linear to spatulate, 1–5 cm. long, 0.1–0.4 cm. wide, acuminate to rounded at apex, short petioled, inflorescence usually glandular-pubescent; buds nodding, 1–2.5 cm. long, acute; hypanthium slender, 4–9 mm. long, with or without a slight swelling at top of ovary, green without, usually purple within, inner ring of hairs about one-

third way from base; calyx lobes green to rose, 0.5–1.5 cm. long, united in anthesis, the tips not free in bud; petals lavender to lilac, rather broadly cuneate, 1–2.8 cm. long, 0.8–2.6 cm. wide, rounded to retuse at apex, not clawed; filaments slightly flattened, 2–8 mm. long, unequal, long ones twice the length of short ones, anthers subequal, 2–7 mm. long, yellow to violet, fertile to tip; style 1.6–3 cm. long, extending to about half way between tips of long stamens and apex of petals; stigma lobes linear, 2–3 mm. long, white or yellow; capsule 1–3 cm. long, rounded at base and borne on pedicel 2–7 mm. long, tapering to slender beak 3–6 mm. long, 8-ribbed, in dried specimens appearing quadrilateral with a small nerve showing in each intercostal space, more or less pubescent with tiny pin-head glands; seeds dark brown, 1–5 mm. long, cellular-pubescent, cresting one-fourth as long as seed proper.

Type locality, not given.

Material examined, CALIFORNIA: Butte Co., Chico, Mrs. Bidwell in 1885 (G); Rancho Chico, Mrs. Bidwell in 1878 (G); El Dorado Co., near Placerville, K. Brandegee in 1907 (C); Sportsman's Hall, 12 mi. above Placerville, K. Brandegee in 1907 (C); Sweetwater Creek, K. Brandegee in 1908 (C); between Coal and Auburn, K. Brandegee in 1908 (C); Amador Co., Armstrong's Station, Hansen 1090, type collection *G. hansenii* (C,G); near Wylie's Station, K. Brandegee in 1910 (C); Pine-Grove, Hansen 1157, in part (C); Calaveras Co., near Harmon Peak, Davy 1407 (C); Mariposa Co., Benton Mills, Congdon in 1892, in part (C); Mariposa Valley, Congdon in 1897 (C); Sherlock's, Congdon in 1898 (C); Mariposa, Congdon in 1897 (S); Lewis, Congdon in 1896 (G); Mormon Bar, Congdon in 1896 (G); without locality, Fremont p.p.p. in 1846, cited as typical material *G. hispidula* (G), Rattan 32 (G), Pratten (G), Mrs. Austin in 1896 (C).

The identity of *Oenothera arcuata* Kellogg, Proc. Cal. Acad. 1:58. 1854–57, is not clear. The description more nearly fits *G. amoena* than anything else. As original material does not seem to be extant, there is no way of checking this. It is not certain therefore that JEPSON's *Godetia arcuata* is the same concept as KELLOGG's *Oenothera arcuata*. I have compared material cited by JEPSON as *G. arcuata* with type material of *G. hispidula* and of *G. hansenii*, and regard them as identical. All have the long-beaked capsule and glandular pubescence. The annular swelling at top of ovary (which is the chief character JEPSON used to designate *G. hansenii*) is present in all

of them, and may or may not be conspicuous; in fact, part of WATSON's material of *G. hispidula* (the *Pratten* specimen) has a more accentuated swelling than the type of *G. hansenii*. In all of them the inner ring of hairs on the hypanthium is borne well down toward the base.

This is the only species in the genus which has glandular pubescence, and this character is of value in distinguishing *G. hispidula* from *G. dudleyana* and *G. biloba*, which sometimes resemble it superficially.

8. *Godetia cylindrica* (Jepson) comb. nov.—*Godetia bottae* var. *cylindrica* Jepson, Univ. Cal. Pub. Bot. 2:332. 1907.

Erect, slender, simple or branching in upper half; stems 1-5 dm. tall; leaf blades narrowly lanceolate to linear, 2-4 cm. long, 0.15-0.4 cm. wide, acute to rounded, petioles 0.3-1.5 cm. long; inflorescence strigillose; buds nodding, slender, 1-2.5 cm. long, acuminate; hypanthium slender, 1.5-6 mm. long, green to lavender on outside, purple inside, with inner ring of hairs one-third to three-fourths way from base; calyx lobes green tinged with purple, to purple, 1-1.5 cm. long, united in anthesis, the tips not free in bud; petals lavender in upper half, shading to yellow-white, base purple, occasionally lavender or even white, usually marked with small purple dots, cuneate to broadly obovate, 0.8-2.6 cm. long, 0.7-2.3 cm. wide, narrowed at base but not forming a true claw, broadly obtuse or rounded to truncate at apex, the margin entire or slightly incised; filaments flattened, unequal, 2-6 mm. long, lavender; anthers subequal, 4-6 mm. long, yellow to lavender; style equaling or slightly exceeding longer stamens; stigma lobes narrowly ovate, 2-2.5 mm. long and one-third as wide, white to purple; capsule linear, 1.5-4 cm. long, 0.1-0.2 cm. wide, sessile or with pedicel as much as 4 mm. long, tapering to a pronounced beak 2-6 mm. long, 8-ribbed, the ribs alternately heavy and light, easily seen in young capsules, in older specimens and in dried material the capsules appear perfectly smooth and terete, or occasionally quadrilateral; seeds about 1 mm. long, dark brown, cresting not very prominent.

Type locality, Wortham Creek, near Alcalde, Fresno Co., California.

Representative material, CALIFORNIA: Los Angeles Co., Newhall Tunnel, *C. L. Hitchcock* 1 (P); Bouquet Canyon, *Munz* 6926 (P); 5 mi. W. of

Elizabeth Lake, *C. L. Hitchcock* 55 (P);  $1\frac{1}{2}$  mi. S. of Lebec, *C. L. Hitchcock* 44 (P); Kern Co., vicinity of Fort Tejon, *Parish* 1899 (G,S), *Hall* 6279 (C,P); Keene, *Feudge* 1174 (P), *Jones* in 1903 (P); Bakersfield, *Davy* 1713 (C,P); Caliente, *Coville* and *Funston* 1099 (C,S), *K. Brandegee* in 1905 (C); Lower Kern River Canyon, *Abrams* 11969 (P,S); Blue Mt., *Hall* and *Babcock* 5007 (C,P); Ventura Co., Hopper Creek Canyon, *C. L. Hitchcock* 43 (P); Santa Barbara Co., Orcutt, *Jones* in 1926 (P); Gaviota Pass, *C. L. Hitchcock* 2 (P); San Luis Obispo Co., Arroyo Grande, *Cook* in 1895 (C); Cuesta Pass, *C. L. Hitchcock* 6 (P); Santa Margarita, *Mrs. Summers* (C); southern part of San Luis Obispo Co., *Mrs. Summers* 329 (C); Paso Robles, *K. Brandegee* in 1911 (C), *Benjamin Cobb* 16 (C); Fresno Co., King's River, *Duncan* in 1923 (S); Tulare Co., Kaweah River basin, *Hopping* in 1901 (C).

This species shows considerable variation between the northern and southern forms. In its extreme northern range, toward the coast, the flowers have a shortened hypanthium, with the ring of hairs close to the summit, so that it is hard to tell it from *Godetia bottae* var. *typica*, except by the markings of the petals, the 8-ribbed, linear capsule, and narrow leaves. The following numbers are of this nature: San Luis Obispo Co., Templeton, *Wiggins* 2083 (S), *Sweet* in 1919 (S); Monterey Co., between King's City and Jolon, *K. Brandegee* in 1908 (C,G); Santa Lucia Mts., *Dudley* in 1901 (S). In general, it might be said that *G. bottae* as a species is coastal, while *G. cylindrica* is found usually toward the interior. In its southern range, *G. cylindrica* need not be mistaken for either *G. bottae* or *G. dudleyana*, because of the extremely narrow leaves, linear 8-ribbed capsules, coloration of petals, and, above all, by the slender, elongate hypanthium with the ring of hairs inside well down toward the base. In dried material the capsule usually appears quadrilateral or terete and unribbed, but in living material the ribs are as conspicuous as they are in *G. dudleyana* or in *G. viminea*.

9. GODETIA BOTTAE Spach, Nouv. Ann. Mus. Par. III, 4:393. 1835.

Erect, simple, or branching from base; stems 1.5–8 dm. tall; leaf blades linear to lanceolate, 2–7 cm. long, 0.2–1.5 cm. broad, acute to rounded, petioles 0.3–1 cm. long; inflorescence strigillose; buds drooping, 1–2.5 cm. long, slender to thick, acuminate or merely acute; hypanthium short and broad, 1–4 mm. long, inner ring of hairs borne just below summit, about four-fifths of way from base; calyx lobes lavender to green, 1–2 cm. long, united in anthesis, or

reflexed in pairs, tips not free in bud; petals lavender, sometimes lighter toward base, with or without tiny purple dots, cuneate-obovate, 1-3.5 cm. long, 1-3 cm. wide, retuse to obtusely pointed at apex, the margin more or less retuse, rather abruptly narrowed at base to a claw 0.5-1 mm. long; filaments flattened, unequal, 4-10 mm. long, short ones about two-thirds length of long ones; anthers subequal, 4-8 mm. long, yellow or lavender; style equaling or slightly exceeding longer stamens; stigma lobes white to purple, lobes 2 mm. long, or a little less, and one-third as broad; ovaries often canescent and reflexed when young; capsule elongate, 1.5-5 cm. long, 0.2-0.3 cm. thick, terete with no ribbing showing when young, but becoming square in cross-section at maturity and in dried material, at which time a median nerve is sometimes apparent on each face, beakless or with beak 1-4 mm. long, pedicel 0.2-3 cm. long; seeds dark brown, 1 mm. long.

#### Key to varieties

- A. Leaves 0.2-0.4 cm. wide; calyx lobes usually rose tinted. In California from Gaviota Pass, Santa Barbara Co., northward to Monterey Co., along the coast. . . . . 9a. *G. bottae* var. *typica*  
A. Leaves 0.4-1.5 cm. wide; calyx lobes usually green. Gaviota Pass to Orange Co., coastal. . . . . 9b. *G. bottae* var. *deflexa*  
9a. *GODETIA BOTTAE* var. *typica*, nom. nov.—*Godetia bottae* Spach *loc. cit.*; Jepson, Univ. Cal. Pub. Bot. 2:330. 1907; *Oenothera bottae* T. and G. Fl. N. Amer. 1:505. 1840; Watson, Proc. Am. Acad. 8:599. 1873; Leveille Monog. Onoth. 270. 1908, in small part, according to specimens cited; *Oe. godetia* Steud. Nom. ed. II, 2:206. 1841.

Calyx lobes usually rose-colored; leaves narrow, 1.5-4 cm. long, 0.2-0.4 cm. wide; petals 1.2-2.5 cm. long, 1-2 cm. wide; young ovaries erect or deflexed, rather densely pubescent but not silvery.

Type locality, Monterey, California.

Representative material, CALIFORNIA: Monterey Co., Carmel Bay, Heller 6807 (G,S), Heller 4782 (C,P); Pacific Grove, Heller 6757 (C,G,P,S); Seaside, Eastwood 135 (G); Monterey, Bolla in 1829, type material (G), Guirardo 701 (G), Parry (G); Santa Lucia Mts., Dudley in 1903 (S); Bardino, Elmer 4645 (C,P,S); near Monterey, K. Brandegee in 1908 (G,P,S); Monterey, C. L. Hitchcock 10 (P); San Luis Obispo Co., San Luis Obispo, Palmer 145 (C,G); Paso Robles, Barber in 1899 (C); California, Hartweg 1729 (G); Brewer 701 (G).



9b. *GODETIA BOTTAE* var. *deflexa* (Jepson) comb. nov.—*Godetia deflexa* Jepson, Univ. Cal. Pub. Bot. 2:332. 1907.

Sepals usually green, seldom rose-tinted; leaves wide, 2–7 cm. long, 0.4–1.5 cm. wide; petals 1.6–3.5 cm. long, 1.5–3 cm. wide; young ovaries deflexed, silvery-canescens; plants larger and more robust than in the typical variety.

Type locality, "sandy plains of Los Angeles."

Representative material, CALIFORNIA: Santa Barbara Co., Gaviota, *Elmer* 3848 (C,G,S,P); Surf, *K. Brandegee* in 1909 (C); Los Angeles Co., Ravena, *Craig* 495 (P); Santa Monica Mts., Sepulveda Canyon, *Abrams* 2553 (C,G,S,P), Cold Water Canyon, *Munz* and *Harwood* 3927 (P,S), Beverly Hills, *C. L. Hitchcock* 50 (P); Mt. Lowe, *Grant* 6666 (C,S); San Gabriel Canyon, *Eastwood* 8958 (G); Elysian Park, *Brandegee* and *Braunton* 124 (C,G,P,S); San Dimas Canyon, *C. L. Hitchcock* 51 (P); San Bernardino Co., Santa Ana River Canyon, *Munz, Street, Williams* 2647 (P,S), *C. L. Hitchcock* 49 (P). The following approach the typical variety, the leaves being fairly narrow and young ovaries pubescent: Santa Barbara Co., Lompoc, *Hoffmann* in 1927 (SB); Ventura Co., Red Reef Canyon, Topatopa Mts., *Abrams* and *McGregor* 132 (G,P,S); Conejo Grade, *Abrams* 6550 (S); without locality, hills bordering the Mohave Desert, *Pringle* in 1882 (G); Coast Hills, *Lemmon* 247 (G); Santa Barbara Co., *Torrey* 110 (G).

I have seen a photograph of the type of *G. deflexa* and consider it exactly like the collection from Gaviota, *Elmer* 3848, which JEPSON says is perhaps the same as *Cobb's* collection (the type). All of the material south of Gaviota Pass is considered to be *G. deflexa*. I do not, however, consider *G. deflexa* specifically distinct from *G. bottae*. Both have terete, smooth capsules when immature, and quadrilateral, nerved capsules when dry. They both may have the ovaries deflexed before anthesis, although the var. *deflexa* shows this tendency more often than the var. *typica*. Aside from the fact that the var. *deflexa* is the more robust plant and has wider leaves, there is little observable difference between the two.

10. *GODETIA DUDLEYANA* Abrams Fl. L. A. and Vic. 267. 1904.—*Godetia dudleyana* Jepson, Univ. Cal. Pub. Bot. 2:333. 1907; *Clarkia dudleyana* Macbr., Contr. Gray Herb. n. ser. 56:54. 1918; *G. bottae* var. *usitata* Jepson, Univ. Cal. Pub. Bot. 2:332. 1907; *G. jucunda* Jepson, loc. cit. 334.

Erect, usually branching from middle, occasionally simple; stems

1.5-7 dm. tall; leaf blades narrowly lanceolate to spatulate, 2-5 cm. long, 0.3-1.1 cm. wide, acute to rounded, with petioles 0.3-1.2 cm. long, finely pubescent; inflorescence strigillose; buds drooping, slender, 1-2 cm. long; hypanthium short, 1-4 mm. long, lavender to green within and without, ring of hairs within, at, or near summit; calyx lobes 0.6-1.5 cm. long, lavender, united in anthesis, the tips not free in bud; petals cuneate, pink to deep magenta shading to white at base, usually with crimson or purple dots in lower portion, 1-3 cm. long, 0.7-2 cm. wide, truncate to rounded at apex, the margin entire to retuse, tapering uniformly to a short claw 0.5-2 mm. long; filaments filiform, subequal, 4-8 mm. long; anthers subequal, 5-7 mm. long, those opposite petals lavender at base, yellow toward tip, dehiscent first, alternate ones lavender, or all anthers yellow; style equalling longer stamens or exceeding them by as much as 3 mm.; stigma lobes 1-2 mm. long, one-third to one-half as broad, yellow to lavender; capsule slender, 1-3 cm. long, 0.1-0.3 cm. thick, beakless or tapering to a beak, 1-4 mm. long, rounded at base, usually sessile, or with pedicel 1-5 mm. long, 8-ribbed, terete, in dried material often appearing quadrilateral and with ribbing somewhat obscure; seeds about 1 mm. long, dark brown, creasing minute.

**Type locality,** Little Santa Anita Canyon, San Gabriel Mts., California.

**Representative material,** CALIFORNIA: Riverside Co., San Jacinto Mts., *Hall* 2265 (C,P); San Bernardino Co., Bluff Lake, *Williams* (C); San Bernardino, *Parish* 5054 (P), *Parish* 3672, type *G. bottae* var. *usitata* (C,G); Redlands Hills, *Greata* 536 (C); Los Angeles Co., Evey's Canyon, near Claremont, *C. L. Hitchcock* 45 (P,G); San Dimas Canyon, *C. L. Hitchcock* 46 (P); Puddingstone Canyon, San Dimas, *Munz* 10814 (P); Wilson's Trail near Santa Anita Canyon, *Abrams* 2625, type collection (C,G,S); Verdugo Canyon, *Macbride* and *Payson* 756 (G); Kern Co., Poso Creek Valley, *Dudley* 564 (S); Inyo Co., Owen's Valley, *Austin* 387 (C); Tulare Co., Sequoia Nat. Forest, *Dudley* 1796 (S); Old Colony Mill, *K. Brandegee* in 1907 (C,P); valley of Kaweah R., *Coville* and *Funston* 1320 (C); Middle Tule R., *Purpus* 5036 (G); Fresno Co., Converse Basin, *Dudley* 3400 (S); between Pine Ridge and Toll House, *K. Brandegee* in 1910 (C); Madera Co., Raymond, *Cummings* in 1896 (G); Mariposa Co., Mariposa, *Congdon* in 1896 (G,S); Merced Canyon, *Abrams* 4681 (C,G,P,S); Tuolumne Co., Tuolumne River, *Blasdale* in 1895 (C). The following collections are peculiar in that the capsules are unusually heavily ribbed: San Antonio Canyon, near Claremont, *Baker* 3665 (G,P); Los Angeles Co., Azusa, *Nevin* 32a (G); Tulare Co., So. Fork Kaweah R., *Eastwood* 4495 (G,P).

This species has been confused with *G. bottae*, *G. cylindrica*, and *G. hispidula*. It is distinguished from *G. bottae* by the slender, 8-ribbed capsule and filiform filaments, *G. bottae* having a smooth capsule and flattened filaments. In fresh material *G. dudleyana* has a round capsule with eight conspicuous ribs. When dried its capsule often appears square in cross-section and the ribs are not easily seen. *G. dudleyana* is readily distinguished from *G. cylindrica* and *G. hispidula* by the position of the ring of hairs on the inside of the hypanthium. The last two species bear this ring near the middle of the tube, while *G. dudleyana* has it at the top. Furthermore, the red ring at the base of the petals in *G. cylindrica* is distinctive and the glandular pubescence of *G. hispidula* easily separates it.

Comparison of type material of *G. dudleyana* with *Parish* 3672, the type of *Godetia bottae* var. *usitata*, shows that they are identical. Furthermore, there is no difference between the material from Southern California and that of the Sierras; therefore *G. jucunda*, a name which JEPSON proposed for the Sierran plants, must be treated as a synonym. The lobing of the stigma in *G. dudleyana* is worthy of special note, varying considerably. Variation is found in the type collection, and often on the same plant different flowers show a wide range in the length of the lobes.

11. GODETIA EPILOBIOIDES (Nutt.) S. Wats., Bot. Calif. 1:231. 1876.—*G. epilobioides* Jepson, Univ. Cal. Pub. Bot. 2:343. 1907; *Oenothera epilobioides* Nutt. in T. and G. Fl. N. Amer. 1:511. 1840; Wats., Proc. Am. Acad. 8:599. 1873; Leveille Monog. Onoth. 262. 1908; *Sphaerostigma epilobioides* Walp. Rep. 2:78. 1843; *Clarkia epilobioides* Nels. and Macbr., Bot. Gaz. 65:60. 1918; *C. modesta* Jepson Man. Fl. Pl. Cal. 673. 1925.

Erect, simple to diffusely branching entire length but usually sparingly branched from near middle; stems 1.5–4.5 (–6.5) dm. tall; leaf blades linear to broadly lanceolate or spatulate, 1–4 cm. long, 0.2–0.6 cm. broad, acute to blunt, petiole 2–6 (–9) mm. long; inflorescence strigillose; buds nodding, 5–8 mm. long; hypanthium very short, 1–1.5 mm. long, inner ring of hairs near summit; calyx lobes united in anthesis, 3–5 mm. long, tips not free in bud; petals obovate, 6–11 mm. long, 4–8 mm. wide, commonly whitish although sometimes pink or lavender, this color often accentuated when dried,

with or without purple spots near base, rounded at apex, tapering to a very short claw; filaments unequal, 2-6 mm. long, filiform, short ones about two-thirds length of long ones; anthers subequal, 1-1.5 mm. long, yellow or white; style about equaling long stamens; stigma lobes very short, not over 0.5 mm. long, yellow; capsule linear, 1.5-3 cm. long, 0.1-0.15 cm. thick, rather densely strigillose when young, sparsely so in age, weakly 8-ribbed, appearing quadrilateral in pressed specimens, beak 0.5-2 mm. long, pedicel 1-10 mm. long; seeds dark brown, 0.5 mm. long and two-fifths as broad, cellular-puberulent, cresting minute.

Type locality, San Diego, California.

Representative material, CALIFORNIA: San Diego Co., San Diego, Nuttall, type collection (G); Mission Hills, Abrams 3442 (G,P,S); near Fallbrook, Abrams 3331 (C,G,P,S); Tecate Mt., Munz 8005, petals yellow to lavender (P); Ramona, K. Brandegee in 1894 (C); Riverside Co., Glen Ivy, Munz 5052 (P); San Jacinto Mts., Mrs. Gregory in 1892 (C); Banning, Gilman 23 (C); Box Springs Mt., Hall 273 (C); San Bernardino Co., San Bernardino, Parish in 1901 (S); Santa Ana River Canyon, Munz, Street, Williams 9439 (P); Arrowhead Hot Springs, Grant in 1906 (S); Los Angeles Co., Topanga Canyon, Munz and Harwood 3984 (P); Santa Monica Mts., Santa Inez Canyon, C. L. Hitchcock 48 (P); Pasadena, Allen in 1895 (G); Glendale to Burbank, Braunton 887 (C,S); Claremont, Baker 4735 (C,P); San Gabriel Canyon, Munz 9439 (P); Santa Catalina Island, K. Brandegee (C); Orange Co., Laguna, Munz 2175 (P); Ventura Co., Casitas Pass, Hall 3138 (C,P,S); Santa Barbara Co., Santa Cruz Island, Hoffmann in 1925 (SB); San Marcos Road, Painted Cave Ranch, Eastwood 43 (C,G); San Luis Obispo Co., Arroyo Grande, King in 1895 (C); Monterey Co., Santa Lucia Mts., Plaskett 134 (G); Tassajara, Dudley in 1901 (S); San Benito Co., between Emmet and Panoche Pass, Abrams and Borthwick 7886 (S); New Idria, Abrams and Borthwick 7970, flowers pink (S); Hernandez, Lathrop in 1903 (S); Fresno Co., below Huntington Lake, A. L. Grant 1160 (S); Santa Clara Co., near Bald Peak, Dudley 4198, petals pale lavender (S); Contra Costa Co., Antioch, K. Brandegee (S); Stanislaus Co., Knight's Landing, Bigelow in 1853-4, petals pink (G); Mariposa Co., Lewis, Congdon in 1895, petals lavender (S); Mormon Bar, Congdon in 1883 (G); LOWER CALIFORNIA: Guadalupe Mts., Orcutt 858 (G); San Requis, T. S. Brandegee in 1889 (C).

The type of *Clarkia modesta*, which was kindly lent by JEPSON, matches in all respects other material from Central California, such as Heller's 11458 from 10 miles east of Alder Springs, Glenn Co. (G,S), which I consider to be *G. epilobioides* (cf. Munz and Hitchcock, Bull. Torr. Club. 56:197. 1929).

12. GODETIA BILOBA (Durand) S. Wats., Bot. Calif. 1:231. 1876. —*G. biloba* Jepson, Univ. Cal. Pub. Bot. 2:333. 1907; *Oenothera biloba* Durand, Jour. Acad. Nat. Sci. Phila. II, 3:87. 1855; S. Wats., Proc. Am. Acad. 8:599. 1873; *Clarkia biloba* Nels. and Macbr., Bot. GAZ. 65:60. 1918; *Oe. prismatica* var. *biloba* Leveille Monog. Onoth. 264. 1908; *G. dudleyana* f. *brandegeae* Jepson, Univ. Cal. Pub. Bot. 2:334. 1907.

Erect, simple or branching above or from base; stems 2–7.5 dm. tall; leaves elliptic to linear-elliptic, 1–5 cm. long, 0.1–0.5 cm. wide, acute, petiole 0.5–1.5 cm. long; inflorescence strigillose; buds drooping, 1–2 cm. long, with slender point 1–2 mm. long; hypanthium 1.5–5 mm. long, usually tinged with red, tips not free in bud; petals magenta, with or without purple dots near base, narrowly to broadly cuneate, 1–2.3 cm. long, 0.4–1.8 cm. wide, with claw 1–2 mm. long, the apex exceedingly variable, from slightly retuse to deeply so, the petal then forming a deep V with the two lobes occasionally in turn slightly divided; filaments subequal, 4–8 mm. long, subfiliform; anthers 4–7 mm. long, yellow to lavender; style equal to or slightly exceeding longer stamens; stigma rather shallowly lobed, lobes 1–1.5 mm. long and half as broad, lavender; capsule rather short, 1–2.5 cm. long, 0.15–0.2 cm. thick, borne on pedicels 0.1–1 cm. long, beakless or short beaked, 8-ribbed, terete but in dried material often apparently quadrilateral; seeds 1 mm. long, dark brown, creasing minute.

Type locality, Nevada City, California.

Representative material, CALIFORNIA: Contra Costa Co., Port Costa, K. Brandegee in 1905 (C), C. L. Hitchcock 15 (P); between Port Costa and Martinez, K. Brandegee in 1907 (C), C. L. Hitchcock 13 (P); Mt. Diablo, Elmer 4319 (C,P,S); Mariposa Co., Mariposa, Congdon 187 (S); Mt. Bullion, Yosemite National Park, Bolander and Rattan 6364 (C,G); Tuolumne Co., Table Mt., A. L. Grant in 1915 (S); Rawhide Hill, Mrs. Williamson 68 (P,S); Groveland, Abrams 4708 (G,P,S); Calaveras Co., Angel's Camp, Davy 14843 (C); near Mokelumne Hill, Rattan in 1885 (G,S); Amador Co., Near Jackson, Mullikin 108 (C,P,S), C. L. Hitchcock 29 (P); between Sutter Creek and Pine Grove, C. L. Hitchcock 33 (P); El Dorado Co., Sweetwater, K. Brandegee in 1907 (G,P); Nashville, Rixford in 1902 (C,G); Placer Co., Colfax, Jones 3340 (P,S), Eastwood 619 (G); 4 mi. E. of Colfax, Heller 12746 (G,S); Nevada Co., near Wolf Creek, Eastwood 3437 (G); California, ex Durand 1855, probably type material (G).

Some of the Sierran plants of *G. biloba* approach *G. dudleyana* quite closely, the petals being very shallowly retuse. Such collections as those from Nevada Co., Bear River, *Hall* 10155 (C,P); Mariposa Grove, *King* in 1907 (C); and El Dorado Co., Simpson's Ranch, Sweetwater Creek, *K. Brandegee* on May 29, 1907, type of *G. dudleyana* f. *brandegeae* (C), are of this nature. In such cases there are several characters that help to distinguish the two. The capsule in *G. dudleyana* is sessile or very short pedicelled, while in *G. biloba* the pedicel may be as much as 1 cm. long. The stigma lobes are shorter in *G. biloba*, and the bud is more nearly ellipsoid and has a longer point than that of *G. dudleyana*.

#### Doubtful or excluded species

1. *OENOTHERA HUMILIS* Donn Cat. Hort. Cantab. ed. I, 41. 1796.—The only reference to this plant which I can find is a footnote upon the page on which *Oe. purpurea* is described (B. M. 352, Bot. Mag. 10:t. 352. 1796). Mr. T. A. SPRAGUE of Kew kindly supplied the following information:

"We do not have the 1st edition of Donn's Hort. Cantab. at Kew, nor is it represented in the British Museum (Natural History). In the Kew Herbarium, however, there is a letter from the late Dr. JACKSON according to which the publication of *Oenothera humilis* was as follows: 'humilis. W. Coast N. Am. 1791. July. H. O.' (Donn Hort. Cantab. ed. I, 47\* (?): 1796.) In my opinion and in that of my colleagues the information accompanying each name in the Hort. Cantab. was not intended for a description and cannot be accepted as such. Roxburgh's *Hortus Bengalensis* is a parallel case (see Kew Bull., 1925, pp. 311-315)."

2. *OENOTHERA ROMANZOWII* Ledeb. ex Hornem., Hort. Hafn. Suppl. 133. 1819.—This species is apparently known only from garden specimens, the description being drawn from plants grown from seeds collected by *Chamisso* on the northwest coast. In the Gray Herbarium there are two sheets, one from the "Jardin des plantes, le 3 aout 1837," and one labeled "Cult. Sartwell, e sem. Hort. Soc. Lond. 1833," which I take to be typical material. These plants resemble the material of *Oe. tenella* from South America more closely than anything else which I have seen. In Mrs. BRANDEGEE'S notes is

this memorandum: "Lindley, in Hort. Trans. VI. 94 (1827) says: 'It is not improbable that the *Oe. romanzowii* of Hornemann and the Continental Gardens may be the same as *Oe. tenella*, but the *Oe. romanzowii* published in the Botanical Register, t. 562 is not different from *Oe. purpurea*. I had many opportunities of observing the plant which was in 1821 called *romanzovii*, both in the Chelsea Garden, and in Mr. KENT's garden at Clapton, whence the figure for the Botanical Register was obtained, and I have no difficulty in declaring that it was scarcely distinguishable even as a variety from *Oe. purpurea*.'"

The plate in the Botanical Register does resemble *G. purpurea* very closely, but the capsules in the illustration are much larger and thicker than in the two collections which I have seen.

3. GODETIA NIVERTIANA Goujon in Rev. Horticole 431. 1872.—This is a *nomen nudum*, published in a horticultural journal, without diagnosis.

4. GODETIA PULCHERRIMA Greene, Pittonia 2:217. 1891.—This would seem from its description to be *G. bottae* var. *deflexa*. The type from Los Angeles Co., Mrs. W. F. Wheeler, I am unable to locate. Mr. Parish's n. 1899 from Fort Tejon, which was "apparently about the same," is undoubtedly *G. cylindrica*, but it certainly does not have "petals . . . lilac and streaked with white veins above, whitish at the base," nor a distinctly pedicelled capsule. Apparently the two collections are not conspecific and because of this ambiguity the name is rejected.

5. GODETIA DELICATA Abrams, Bull. Torr. Bot. Club 32:539. 1905 is to be referred to *Clarkia* (cf. Munz and Hitchcock, Bull. Torr. Club 56:188. 1929).

6. GODETIA LATIFOLIA Nels. and Ken., Proc. Biol. Soc. Wash. 19:156. 1906; type from Sierra Co., Sierra Valley, California, Miss Helen Hamlin, July, 1904. Through the kindness of Professor FLEMING of the University of Nevada, this type has been made available. It is typical *Clarkia rhomboidea*.

POMONA COLLEGE  
CLAREMONT, CALIF.

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NEW OR OTHERWISE NOTEWORTHY  
COMPOSITAE. IV

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 403

EARL EDWARD SHERFF

*Bidens distans* sp. nov.—Frutex erectus, glaber, ramosus, verisimiliter 6–9 dm. altus, caule subtereti, glaucescenti. Folia petiolata petiolis tenuibus 3–4.5 cm. longis, petiolo adjecto 9–16 cm. longa, nunc pinnatim 3–5-partita foliolis lanceolatis serratis dentibus acriter mucronatis, apice acutis sed parce acuminatis; nunc indivisa lamina lanceolata  $\pm$  11.5 cm. longa et  $\pm$  4 cm. lata, unico latere circ. 16–20 dentibus munita. Capitula paniculato-corymbosa, numerosa, subdense disposita (distantia) pedicellis tenuibus plerumque 1–2 cm. longis, radiata, pansa ad anthesin 1.5–2 cm. lata et circ. 6–7 mm. alta. Involucri bracteae exteriores 4–6, oblongo-lineares, obtusae, minimae (1–2 mm. longae), quam interiores lanceolatae multo minores. Flores ligulati plerumque 5, ligula anguste oblongi, flavi, apice plus minusve denticulati, 8–12 mm. longi. Achaenia recta vel vix curvata, anguste linearia, plana, exalata, atra vel ad apicem brunnea, unaquaque facierum circ. 4-sulcata, corpore 8–11.5 mm. longa et 1.1–1.7 mm. lata, facie marginibusque glabra, apice interdum paucisetosa, sub apice irregulariter biaristata aristis erectis et retrosum barbatis, usque ad 1 (rarius –3) mm. longis.

**Specimens examined:** *Charles N. Forbes* 148L, "at Gay's," on mountains near Koele,<sup>1</sup> north of Maneke Bay, Isl. Lanai, Hawaiian Isls., June, 1918 (type, Herb. Field Mus.: cotype, Herb. Bishop Mus.); *G. C. Munro*, Kapano, Isl. Lanai, Jul. 28, 1915 (Herb. Field Mus.).

Differs from *B. hawaiiensis* Gray in its smaller and more numerous heads, minute exterior involucral bracts, fewer rays, mostly divided leaves, etc. Differs from *B. micrantha* Gaud. in its more open inflorescence, dullish-black not truly shining achenes, glaucescent stem, etc.

*BIDENS TENERA* var. *paucidentata* (O. E. Schulz) comb. nov.;

<sup>1</sup> Spelled Koala upon United States Geological Survey map. But a Mrs. GREENLAND (née GAY) informed *H. Wiebke* (fide *O. Degeneri in litt.*, Feb. 9, 1928) that the name is spelled locally Koele, not Koala.



*B. ekmanii* et var. *paucidentata* O. E. Schulz in Urban, Repert. Spec. Nov. Regn. Veg. 26:III. 1929; *B. tenera* var. *tetracera* Sherff, Bot. GAZ. 88:293. 1929.—My *Bidens tenera* var. *tetracera*, published in November, 1929, was found to have been antedated several months by the equivalent *B. ekmanii* O. E. Schulz and its so-called var. *paucidentata*, both of Cuba. The type specimens of *B. ekmanii* and its var. *paucidentata* are now before me (through the kindness of Dr. GUNNAR SAMUELSSON, Director of the Botanical Section of the Natural History *Reichsmuseum* of Stockholm). They differ mutually to an insignificant extent. Nor are they separable from the South American specimens described by me for *B. tenera* var. *tetracera*. The varietal name *paucidentata* accordingly is here taken up as required by the Vienna Code.

*Bidens townsendii* sp. nov.—Herba annua, erecta, subsimplex, 8-10 dm. alta, caule acriter tetragono glabra. Folia tenuiter petiolata petiolis usque ad 2.5 cm. longis, petiolo adjecto circ. 8-10 cm. longa, 1-2-pinnata, segmentis primariis lateralibus circ. 2 jugis, superioribus simplicibus inferioribus saepius tripartitis, segmentis lanceolatis membranaceis acriter serratis adpresse setosis, apice acuminatis, sub medio circ. 0.7-1.7 cm. latis. Capitula pauca, tenuiter pedunculata pedunculis 3-6 cm. longis, subradiata, pansa ad anthesin  $\pm$  1.2 cm. lata et circ. 6 mm. alta. Involucri bractee exteriores 8-12, anguste lineares, superne non spathulato-dilatatae, apice acerrimo induratae, 3-6 mm. longae, interiores lanceolatae subaequales. Flores ligulati  $\pm$  5, rudimentarii, ligula obovati, albidi vel subrosacei, plus minusve perspicue striati, circ. 4 mm. longi. Achaenia exalata, exteriora clavata, obcompressa-tetragona, rubidonigra, hinc inde saepe sparsim setosa, corpore circ. 4-4.5 mm. longa et 1-1.2 mm. lata, apice calva vel biaristata, aristis stramineis retrorsum hamosis circ. 0.5 mm. longis; interiora tenuiter linearia, corpore usque ad 11 mm. longa, nigra, supra marginaliter setosa, biaristata aristis stramineis retrorsum hamosis 1-1.5 mm. longis.

Specimens examined: C. H. T. Townsend and C. M. Barber 415, near Chihuahua in the Sierra Madres, State of Chihuahua, Mexico, Sept. 6, 1899 (type, Herb. N.Y. Bot. Gard.).

A close ally of the South American *Bidens subalternans* DC., the Old World *B. biternata* (Lour.) Merr. & Sherff, and the cosmopolitan

*B. pilosa* L. From the last it is readily distinguished by the lack of apical dilation in the exterior involucre bracts.<sup>2</sup>

*BIDENS HAWAIIENSIS* **conglutinata** Degener and Sherff var. nov.—A specie achaeniorum aristas omnino summo corpori conglutinatissimis non distantibus, erectis tenuibus circ. 2–3 mm. longis differt.

**Specimens examined:** *Antone Borges* 3798, on dry lava flow of 1840, south-east of Pahoa, easternmost part of Isl. of Hawaii, Hawaiian Isls., Nov. 18, 1929 (type, Herb. Field Mus., 2 sheets: cotypes, Herb. Kew; Herb. Berl.; Herb. Gray).

*Bidens malmei* sp. nov.—Herba annua, erecta, caule acriter tetragona, supra ramosa, 0.4–1 m. alta. Folia tenuiter petiolata petiolis usque ad  $\pm 4$  cm. longis, petiolo adjecto 4–10 cm. longa, minutissime sparsimque pubescentia, ciliata, membranacea, primaria mediana subbipinnata, foliolis lateralibus 2–3-jugis, basalibus rursus 2–3-partitis, aliis indivisis, omnibus circumambitu ovatis circ. 2.5–3.5 cm. longis, crenato-dentatis, dentibus (saepè duplicibus) unico latere circ. 6–12; superiora minora, bipinnata vel etiam tripinnatisecta, segmentis plus minusve ovato-oblongis. Capitula laxè corymboideo-paniculata, tenuiter pedunculata pedunculis 5–17 cm. longis, subradiata, pansa ad anthesin circ. 6 mm. alta et aequaliter lata. Involucri basaliter hispida bractee exteriores 6–8, lineares, hispidae, apice subacutae, sub apice rarissime subdilatatae, 3–4 mm. longae; interiores lineari-lanceolatae, 5–7 mm. longae. Flores ligulati albidii rudimentarii, ligula ipsa tantum circ. 1–1.5 mm. longi plus minusve obcordati ac apice paucidentati. Achaenia linearia, nigra, tetragona, unaquaque facierum 2-sulcata, glabra vel aegre erecto-setosa, corpore 7–11 mm. longa et 0.5–0.7 mm. lata, apice exteriora 2–3-alia 4-aristata, aristas stramineis retrosum hamosis, 2–3 mm. longis.

**Specimens examined:** *Gust. O. A. Malme* 1456, near Menino Denso, Porto Alegre, State of Rio Grande do Sul, Brazil, March 3, 1902 (Herb. Mus. Bot. Stockholm, 2 type sheets).

Allied with *Bidens bipinnata* L., *B. duranginensis* Sherff, *B. gardneri* Baker, and *B. subalternans* DC.

*Dahlia linearis* sp. nov.—Caules tenues, ramosi, lignescentes, subglabrati, striati, ramorum internodiis circ. 5–6 cm. longis. Folia

<sup>2</sup> Duplicates of the type were widely distributed to herbaria under Prof. E. L. GREENE'S determination, *Bidens heterophylla* Ort. In the main, however, they lacked mature fruiting heads and were omitted in my mss.

opposita, tenuiter petiolata petiolis planis basi connatis ventraliter basaliterque brunneo-pilosis tantum 0.3–2.2 cm. longis, petiolo adjecto 5–8 cm. longa, 2–3-pinnata, segmentis ultimis linearibus (1–2 mm. latis), glabris vel hinc inde praecipue ad venas adpresso-hispidis, apice acuto submucronatis. Capitula subnumerosa, breviter tenuiterque pedunculata, radiata, pansa ad anthesin 4–6 cm. lata et 1.2–1.4 cm. alta. Involucri glabri bractee exteriores 5–8, patentes, oblongo-lanceolatae, dorsaliter 4–5-lineatae, 5–7 mm. longae; interiores oblongo-lanceolatae vel oblongo-ovatae, adpressae, 1.3–1.6 cm. longae. Flores ligulati  $\pm$  7, rosacei, ligula anguste elliptico-obovati, apice mucronulati vel minutissime sed acerrime 2–4-denticulati, 2.2–3 cm. longi. Achaenia clavata, grisea vel atro-grisea, plana, exalata, unaquaque duarum facierum mediano-costata ac circ. 8–10-striata, rugosa, nunc aegre hispida nunc glabrata, 8.5–9 mm. longa et supra circ. 1.2 mm. infra circ. 0.5 mm. lata, apice saepius bidenticulata sed non vere aristata.

**Specimens examined:** *Brother Agniel* (Bro. G. Arsène distrib. no. 10286), alt. 1850 m., Querétaro, State of Querétaro, Mexico, 1910–1913 (type in Herb. Mo. Bot. Gard., herb. no. 841136).

Closest to *Dahlia dissecta* Wats., from which it differs in its more slender and more woody branches, its shorter internodes (which give a more compact habit), its very different leaves (petioles 0.3–2.2 cm. not mostly 3–6.5 cm. long, at their very base densely brownish-pilose not glabrous or nearly so, ultimate segments 1–2 mm. not mostly 4–7 mm. wide, etc.), its more numerous and much shorter peduncles (4–7 cm. not 15–30 cm. long), its oblong-ovate not ovate-orbicular exterior involucre bracts, etc. The two small specimens of the type sheet are each a little more than 2 dm. long. One had 5 heads, the other 9 heads. The two apical teeth found on most of the achenes represent of course the aristae found so commonly in *Bidens*, *Coreopsis*, and *Cosmos*, but normally absent in *Dahlia*.<sup>3</sup>

*Coreopsis heterolepis* sp. nov.—Herba perennis, glabra, caule angulata, ramosa, dense foliosa, 3–6 dm. alta. Folia membranacea

<sup>3</sup> *Dahlia* material cultivated by Prof. J. B. S. NORTON at Hyattsville, Maryland, in 1926, "original source unknown," was found to have achenes biaristate, the aristae naked, almost capilliform, and mostly 6–10 mm. long! Prof. NORTON had already cultivated this peculiar form through three generations at the time of his sending me some ripened heads for study.

plus minusve dimorpha: basalia dense congregata, petiolata petiolis planis angustissime alatis usque ad 6 cm. longis, petiolo adjecto 8-12 cm. longa, indivisa spathulata vel oblonga apice obtusa, alia pinnata foliis nunc linearibus nunc oblanceolatis nunc oblongis nunc rhomboideo-subovatis ac etiam 1-1.7 cm. latis, lateralibus saepius 1-2 jugis ac multo minoribus; caulina numerosissima, petiolata petiolis anguste marginatis marginis basi setis multiloculatis sparsim positis obsitis circ. 1-2.5 cm. longis petiolo adjecto circ. 6-10 cm. longa, plerumque pinnata, foliolis anguste linearibus, 2-8 cm. longis et 0.3-2.5 mm. latis, apice subacutis, lateralibus 1-2 dm. longis, radiata, pansa ad anthesin 3-4 cm. lata et 5-7 mm. alta. Involucri glabri vel subglabri bractee exteriores angustissime lineares, saepius patentés, apice acutae, 5-11 mm. longae; interiores ovato-lanceolatae nunc breviores nunc longiores. Flores ligulati circ. 8, omnino flavi, ligula cuneato-obovati, apice obtuse 4-dentati, circ. 1.5-1.8 cm. longi. Paleae superne capillares, circ. 4-5 mm. longae. Disci florum stigmata caudato-elongata. Achaenia minima, plano-convexa, corpore nigro oblongo vel oblongo-ovato ipso tantum circ. 1.3-1.7 mm. longa et circ. 1-1.5 mm. lata, faciebus levia vel papillato-rugosa (ventrali apicaliter basaliterque saepius callosa), marginibus anguste alata alis rubris circ. 0.2-0.4 mm. latis, apice minutissime 2-squamellata squamellis fimbriolatis.

*Specimens examined:* Ernest Jesse Palmer 6962A, dry, sandy bluffs, Heber Springs, Cleburne County, Arkansas, Oct. 30, 1914 (4 type sheets, Herb. Mo. Bot. Gard.).

*Coreopsis debilis* sp. nov.—Herba perennis, caulibus saepius 2-6, e radice lignescenti erectis, tenuissimis, angulatis, glabris, 3-6 dm. altis. Folia opposita, membranacea, petiolata petiolis tenuibus usque ad 2 cm. longis, lamina nunc indivisa linearia vel parce lineari-oblanceolata margine integra ciliataque apice vix acuta basim versus sensim angustata faciebus saepe aegre hispida petiolo adjecto 2-5 cm. longa et 1-5 mm. lata; nunc majora, petiolo saepe 3.5 cm. longo adjecto 7-9 cm. longa, aegre pinnata (raro etiam subbipinnata) segmentis lateralibus plerumque 1-2 jugis, quam terminali minoribus, linearibus vel subfiliformibus. Capitula pauca vel numerosa, tenuiter pedunculata pedunculis 1-2 (-3) cm. longis, radiata, pansa

ad anthesin circ. 2-2.5 cm. lata et circ. 7-9 mm. alta. Involucris saepius glabrati bractee exteriores circ. 8, lineares lanceolatae, apice acutae vel obtusae, margini ciliatae, basim versus saepe scariosae, 3-4 mm. longae; interiores lanceolatae vel ovato-lanceolatae, 7-8 mm. longae. Flores ligulati flavi, ligula cuneato-obovati, apice saepius 4-dentati dentibus acutis, circ. 1 cm. longi. Paleae lineares, superne angustissime elongatae. Stylorum (florum disci) rami terminaliter caudati. Achaenia obcompressa, tergo papillato-rugoso convexa, corpore ipso atro circ. 2 mm. longa et circ. 1 mm. lata, marginibus anguste alata, apice 2-squamellata squamellis fimbriolatis; facie ventrali glabra vel papillato-scabra, supra infraque valde callosa.

**Specimens examined:** *A. H. Curtiss*, Pensacola, Florida, summer of 1885 (Herb. N.Y. Bot. Gard.); *Charles Mohr*, dry, open, sandy hills etc., *eodem loco*, Jul. 4, 1874 (Herb. U.S. Nat., 2 sheets); *idem*, in woods, Cullman, Alabama, June 1, 1882 (Herb. Gray); *idem*, low, open places, Cullman, June, 1883 (Herb. U.S. Nat., 3 sheets); *idem*, dry hillsides, vicinity of Ashland, Bibb County, Alabama, June, 1883 (Herb. U.S. Nat.); *idem*, borders of woods and fields, dry openings, Mulberry River Valley, Blount County, Alabama, June 6, 1883 (Herb. U.S. Nat.); *idem*, sandy soil in open copses, Columbus, Mississippi, June 4, 1888 (Herb. U.S. Nat.); *idem*, dry, bald prairies, Gallion, Alabama, May 25, 1893 (Herb. U.S. Nat.); *Charles L. Pollard* and *William R. Maxon* 496, Lithonia, Georgia, Aug. 12, 1900 (Herb. N.Y. Bot. Gard.); *John Donnell Smith* 602, dry, sandy old fields along coast of Mississippi Sound, Harrison County, Mississippi, Sept. 15, 1885 (type, Herb. Field Mus.: cotype, Herb. Gray).

A species more closely related to *Coreopsis lanceolata* L. and *C. grandiflora* Hogg. *J. Donnell Smith* had determined his plant as *C. lanceolata* var. *angustifolia* Torr. & Gray. That variety, however, has a very different habit, the plants being less branched and the leaves being basally clustered and almost always simple. *Donnell Smith* had shown ASA GRAY his Mississippi material, and GRAY had called it "a peculiar form" of *C. lanceolata* L. *Mohr's* material from Pensacola, Florida, likewise had been seen by GRAY. Thus, one sheet bears a note stating: "Dr. GRAY remarks: 'I have nothing like this. I suppose it may be an altered, coast form of *C. lanceolata*. But it is very peculiar.'"<sup>4</sup> This same sheet has *Mohr's* first

<sup>4</sup>This note is written on the back of a card which, by strong transmitted light, is seen to have been *Donnell Smith's* regular herbarium label. This would seem to indicate that *Mohr* and his contemporary had studied their several specimens mutually and had regarded them as identical.

determination, *Coreopsis lanceolata* var. *angustifolia*, but this he had subsequently crossed out and replaced with *C. grandiflora*. His other foregoing specimens all had been labeled by him as *C. grandiflora*. This interpretation was retained by him in his Plant Life of Alabama (Contrib. U.S. Nat. Herb. 6:805. 1901). From *C. grandiflora*, however, our plants differ in their more slender and wiry, less herbaceous stems, in the tendency for some entire stems to have minute, simple leaves (smaller than the simple leaves formed in *C. lanceolata*), and in the tiny achenes, which have the body proper about 2 mm. long and about 1 mm. wide, as against about 2.5 mm. long and about 1.4–1.8 mm. wide in *C. grandiflora*.<sup>5</sup>

**COREOPSIS GRANDIFLORA pilosa** var. nov.—A specie caulibus foliisque pubescentibus saepe etiam longe patenteque piloso-hispidis differt.

**Specimens examined:** *Stewardson Brown*, N. L. Britton, and *Peter Bisset* 2011, cultivated, Agricultural Station, Isl. Bermuda, May 22–June 6, 1914 (type, Herb. N.Y. Bot. Gard.); *John H. Kellogg*, cultivated, Missouri Botanical Garden, St. Louis, Missouri, May 23, 1911 (Herb. Mo. Bot. Gard.); *John K. Small*, alt. 700–1000 ft., between Alcovy River and No Business Creek in Oconee and Gwinett Counties, Georgia, Jul. 14, 1893 (Herb. U.S. Nat.).

*C. grandiflora* Hogg is typically glabrous. The two cultivated specimens examined have a very unique aspect because of their marked hispidity. In spontaneous material the hairs are shorter and less spreading.

**COREOPSIS DELPHINIFOLIA** Lam. Encycl. 2:108. 1786; *C. discolor* Link Enum. Hort. Berol. 2:353. 1822.—LINK's description of his own *C. discolor* ("Fol. subsessilibus ternatis foliolis linearibus acutis integerrimis. . . .") would seem to indicate *C. major* var. *rigida* (Nutt.) Boynt. Recently I was very generously lent some specimens from the Museum of the Berlin Botanical Garden, and among these was a sheet of the original cultivated material of *C. discolor*. The plant has the uppermost leaves ternate as described by LINK, but the principal stem leaves each have several more segments than three and place LINK's plant at once with *C. delphinifolia* Lam.

<sup>5</sup> A similar or even more pronounced achenial difference separates *C. debilis* also from *C. lanceolata*. Furthermore, the achenes of *C. debilis* are narrowly winged (0.2–0.5 mm. wide) while those of *C. grandiflora* and *C. lanceolata* commonly have wings 1 mm. or so in width.

*Coreopsis suaveolens* sp. nov.—Frutex ramosus, verisimiliter 5-8 dm. altus, ramis subteretibus plerumque glabris sed hac illac glutinosis odore *Covilleae divaricatae* (Cav.) Vail suaveolentibus. Folia opposita fasciculata, primaria 1.2-1.6 cm. longa, pinnatim 3-5-secta, plus minusve glutinosa, segmentis petiolis similibus vel rursus sectis, ultimis linearibus, glanduloso-hispidis, carnosus, apicaliter mucronatis, tantum 0.3-0.6 mm. latis. Capitula ramos superne nudos (pedunculos tenues) terminantia, solitaria, radiata, pansa ad anthesin  $\pm$  2.3 cm. lata et 7-9 mm. alta. Involucri hispidi bractee exteriores circ. 8, oblongae vel late lanceolatae, obtusae, 3-5 (-7) mm. longae, quam interiores oblongo-lanceolatae circ. 8 mm. longae saepius dimidio breviores. Flores ligulati circ. 6, lutei, ligula oblongo-elliptici, apice integri vel (etiam profunde) bifidi, 7-11 mm. longi. Disci florum stigmata brevia, abrupte incrassata truncataque. Achaenia non visa. Habitu *C. fasciculatae* Wedd. valde similis.

Specimens examined: *Dr. E. Werdermann* 1114, alt. about 3800 m., Cordillera de Lallinca, Province of Tarapacá, Department of Tarapacá, Chile, March, 1926 (type, Herb. Gray: cotypes, Herb. Field Mus.; Herb. Mus. Stockholm; Herb. Univ. California, etc.).<sup>6</sup>

*Coreopsis macbridei* sp. nov.—Frutex glabratus, ramosus, caulis subteretibus. Folia laxè disposita, opposita, petiolata petiolis tenuibus circ. 1-1.5 cm. longis, primaria petiolo adjecto etiam 4-4.5 cm. longa, pinnatim 5-7-secta, segmentis anguste linearibus, glaberrimis, apicaliter acris, 1-2.5 cm. longis et 1-1.7 mm. latis. Capitula corymboideo-paniculata, numerosa, tenuissime pedunculata (vel pedicellata) pedunculis saepius 3-7.5 cm. longis glaberrimis vel apicem versus vix pubescentibus, radiata, pansa ad anthesin tantum circ. 2 cm. lata et 6-9 mm. alta. Involucri glabrati sicci subnigri bractee exteriores circ. 8, lineari-oblongae, obtusae, tantum circ. 1.5-2 mm. longae; interiores oblongo-ovatae circ. 5-6 mm. longae. Flores ligulati circ. 8, fusco-flavi, ligula lineari-elliptici, apicaliter integri vel rarius denticulati, tantum circ. 8-10 mm. longi et 1.5-3 mm. lati. Paleae lineari-oblongae, circ. 5-striatae, tergo villosissimae ventre glabrae, cum achaenio deciduae. Disci florum stig-

<sup>6</sup> The label on the Field Museum sheet says, "Cord. Co. Columfusca, Apacheta, Prov. Tarapacá. . . ."

mata incrassata et minutissime caudato-appendiculata. Achaenia lineari-oblanccolata, valde obcompressa, dorso sub palea glabra, ventre ad costam medianam villosa, marginibus longe perspicueque villosa-ciliata, apice biaristata aristis sursum villosis circ. 2 mm. longis.

Specimens examined: *J. Francis Macbride* 3504, neat compact half-shrub (or shrub) of southwestern rock outcrops, alt. about 7000 feet, Huanuco, Peru, Apr. 26, 1923 (type, Herb. Field Mus.: cotype, Herb. U.S. Nat.); *idem* 3152, ragged shrub, river-canyon slopes, alt. about 7000 feet, Ambo, Peru, Apr. 4, 1923 (Herb. Field Mus.; Herb. U.S. Nat.).

The specimens examined had been determined by Dr. S. F. BLAKE as a form of *Coreopsis townsendii* Blake, with more leaf lobes than on the type. A careful comparison with the type of that species in Field Museum, however, shows many striking differences. *C. macbridei* has a more scraggly habit; its leaves fewer, their petioles narrower and their divisions 5-7 rather than usually 3; the capitula more numerous (more than 30 on one sheet), their peduncles and involucre almost completely glabrous (not conspicuously tomentose), their external bracts about 1.5-2 mm. (not 4-4.5 mm.) long, their diameter at flowering about 2 cm., not 2.5-3 cm., the ligules deep yellow (not light yellow) and only about 8-10 mm. long and 1.5-3 mm. wide, not 15-18 mm. long and 5-7 mm. wide, etc.

*Coreopsis imbricata* sp. nov.—Frutex 6-12 dm. altus, caule ramisque glabris, striatis, ramulorum internodiis numerosis saepius tantum 2-10 mm. longis. Folia opposita, petiolata petiolis tenuibus basi vix connatis circ. 1 cm. longis, petiolo adjecto circ. 2-2.5 cm. longa, ternatim bipinnatisecta, segmentis linearibus, crassiusculis, glabris, eciliatis, acriter apiculatis, 2-8 mm. longis et circ. 0.3-0.6 mm. latis. Capitula ramulos terminantia (vix manifeste pedunculata), radiata, pansa ad anthesin 3.5-4.5 cm. lata et 1-1.2 cm. alta. Involucri villosi bracteae exteriores circ. 16, biseriales, late lineari-oblongae, apice obtusae, 6-7 mm. longae et 1.1-1.5 mm. latae; interiores ovatae, flavo-marginatae, obtusae, 1-1.1 cm. longae. Flores ligulati circ. 8, flavi, ligula oblongo-elliptici, apice integri vel subintegri, 1.8-2.4 cm. longi et 6-7 mm. lati. Paleae lineares, circ. 5-striatae, apice obtusae et denticulatae,  $\pm 8$  mm. longae et  $\pm 0.5$  mm. latae. Disci florum stigmata caudato-apiculata. Achaenia li-



neari-oblancoolata, plana, atra, dorso glabra, ventre ac marginibus ac apice sursum villosa, corpore  $\pm 5$  mm. longa et  $\pm 1.4$  mm. lata, biaristata aristis sursum hispidis  $\pm 2.7$  mm. longis.

Specimens examined: *Mr. R. Pearce*, alt. 12000 ft., Cordillera Huanta (midway between Lima and Cuzco), Peru, February, 1867 (type in Herb. Kew).

Close to *Coreopsis pickeringii* Gray, from which it differs in its numerous, much shorter internodes, its lack of elongate peduncles, the caudate-tipped stigmas of its disk florets, its double outer involucre the bracts of which are larger (with a most noticeable imbricate appearance), etc.

#### Clavis specierum austro-americanorum Coreopsidis

- a. Folia indivisa
  - b. Folia oblanceolata, 5-7 mm. lata.....5. *C. oblanceolata*
  - b. Folia angustiora
    - c. Folia lineari-flagellaria tantum 0.5-1 mm. lata, totam longitudinem aequaliter angusta.....6. *C. longula*
    - c. Folia augustissime spathulato-linearibus, 1-2 mm. lata.....7. *C. venusta*
- a. Folia divisa
  - b. Folia primaria minuta, tantum 7-9.5 mm. longa ramorum internodiis subaequalia.....8. *C. senaria*
  - b. Folia primaria majora
    - c. Capitula pansa ad anthesin circ. 1.1-2 cm. lata; foliorum laminis vel segmentis 1.5-4 mm. latis
    - d. Partes novellae glaucescentia; involucri bracteis exterioribus linearibus quam interioribus dimidio brevioribus.....9. *C. glaucodes*
    - d. Nullae partes glaucescentes
  - e. Involucri bractee exteriores minutae, longitudine tantum circ. tertiae interiorum
    - f. Involucri bractee exteriores ovatae; foliorum segmentis lateralibus cuneatis vel elliptico-obovatis vel spathulatis, 2-6 mm. latis.....10. *C. microlepis*
    - f. Involucri bractee exteriores lineari-oblongae; foliorum segmentis lateralibus anguste linearibus, 1-1.7 mm. latis.....11. *C. macbridei*

- c. Involucri bracteae exteriores lineari-oblongae, quam interiores dimidio breviores.....4. *C. parviceps*
- c. Capitula pansa ad anthesin 2.2-5 cm. lata
- d. Folia densissime congregata, ramis fere abditis
  - e. Foliorum segmenta lineari-oblancoolata, apicaliter sub-obtusa; involucri bracteis exterioribus circ. 8, oblongis, apice rotundatis, circ. 5 mm. longis et 1.5 mm. latis, uniseriatim dispositis.....9. *C. foliosa*
  - e. Foliorum segmenta lineari-subulata, apicaliter acuta; involucri bracteis exterioribus circ. 11, ovatis, circ. 6 mm. longis et 3.3-4.8 mm. latis, biseriatim dispositis.....10. *C. polyactis*
- d. Folia plerumque laxius (*C. trilobae* interdum dense) disposita, ramis plus minusve manifestis
  - e. Foliorum segmenta ultima lanceolata vel oblonga; involucri bracteis exterioribus 2-3 mm. longis et 1-1.6 mm. latis.....11. *C. notha*
  - e. Foliorum segmenta ultima angustiora
    - f. Foliorum triloborum segmenta ultima flagellaria, apicaliter acuta, 1-3.5 cm. longa et plerumque 0.3-0.6 mm. lata
      - g. Folia laxissime patentia vel etiam subreflexa.....12. *C. capillacea*
      - g. Folia dense adgregata, suberecta.....13. *C. triloba*
    - f. Foliorum segmenta ultima diversa
      - g. Involucra glaberrima.....14. *C. spectabilis*
      - g. Involucra (saltem infra) hispida
        - h. Rami foliaque hinc illinc resinosa, suaveolentia.....15. *C. suaveolens*
        - h. Rami foliaque diversa (petiolis raro inferne resinosis)
          - i. Involucri bracteae exteriores biseriales, circ. 16.....17. *C. imbricata*
          - i. Involucri bracteae exteriores uniseriales, multo pauciores
          - j. Foliorum primariorum multa 2.5-4.5 cm. longa

- k. *Achaenia villosa-ciliata* dorso sub palea  
glabra sed ad ventrem villosissima.....  
.....16. *C. pickeringii*  
k. *Achaenia villosa-ciliata* sed faciebus gla-  
bra.....18. *C. townsendii*  
j. Folia primaria 1-1.8 (-2.5) cm. longa.....  
.....19. *C. fasciculata*

Conspectus specierum austro-americanorum *Coreopsisidis*

1. *C. GLAUCODES* Blake & Sherff *ex* Sherff, BOT. GAZ. 80:369.  
1925.—Peru.  
2. *C. MICROLEPIS* Blake & Sherff *loc. cit.* 370.—Peru.  
3. *C. MACBRIDEI* Sherff, *supra* p. 369.—Peru.  
4. *C. PARVICEPS* Blake & Sherff *loc. cit.* 368.—Peru.  
5. *C. OBLANCEOLATA* Blake, Contrib. U.S. Nat. Herb. 22:642.  
1925.—Peru.  
6. *C. LONGULA* Blake, *loc. cit.*—Peru.  
7. *C. VENUSTA* H.B.K. Nov. Gen. et Sp. 4:229. 1820.—Ecuador.  
8. *C. SENARIA* Blake & Sherff *loc. cit.* 367.—Peru.  
9. *C. FOLIOSA* A. Gray, Proc. Amer. Acad. 5:125. 1861.—Peru.  
10. *C. POLYACTIS* Blake & Sherff *loc. cit.* 372.—Peru.  
11. *C. NOTHA* Blake & Sherff *loc. cit.* 373.—Peru.  
12. *C. CAPILLACEA* H.B.K. *loc. cit.* 230.—Ecuador.  
13. *C. TRILOBA* Blake *loc. cit.* 643.—Ecuador.  
14. *C. SPECTABILIS* A. Gray, *loc. cit.*—Peru.  
15. *C. SUAVELOENS* Sherff, *supra* p. 369.—Chile.  
16. *C. PICKERINGII* A. Gray, *loc. cit.* 124; *C. boliviana* Blake, *loc. cit.* 644.—Peru and Bolivia.  
17. *C. IMBRICATA* Sherff, *supra* p. 370.—Peru.  
18. *C. TOWNSENDII* Blake, *loc. cit.* 643.—Peru.  
19. *C. FASCICULATA* Wedd. Chlor. And. 1:71. 1855.—Ecuador  
(where apparently rare) to Peru and Bolivia.

CHICAGO NORMAL COLLEGE

[Accepted for publication March 28, 1930]

## BASICLADIA, A NEW GENUS OF CLADOPHORACEAE

WM. E. HOFFMANN AND JOSEPHINE E. TILDEN

(WITH TWENTY-TWO FIGURES)

Most people are familiar with the term "moss back" as applied to the common snapping turtle, or perhaps have noticed turtles coated with green "moss," in reality green algae. It is surprising to find that little attention or study has been given to the form which lives in this unusual habitat.

Probably the first reference in literature to this subject was a note by GADOW (6) in 1901, concerning snappers in captivity:

Freshwater algae grow on the shell and in the mud which settles on it, and since this happens also in the wild state, they [the turtles] are rendered as inconspicuous as old rotten logs.

A few years later COLLINS (1, 2) described a new species of *Chaetomorpha*, *C. chelonum*, based on collections taken from the backs of turtles, *Chrysemys marginata* and *Aromochelys odorata*, in Michigan. Later COLLINS (3) saw a turtle with a distinct green growth on the shell, at Tewksbury, Massachusetts, and this material proved to be the same species, *C. chelonum*. DITMARS (4) states: "As the snapping turtle is persistently aquatic the shells of many specimens become coated with moss." EVERMANN and CLARK (5) studied the turtles of the Lake Maxinkuckee region, Indiana, and went into considerable detail in notes relating to the algal coating of certain kinds of turtles. Describing *Kinosternon odoratum* (Latreille), they state:

Like the snapper, the musk turtle is frequently covered with algae on the back, the algae often being quite long and thick. The proportion of turtles covered with algae varies with the season and conditions; in early summer, before the scutes were shed, all or nearly all the turtles would probably be covered; with the shedding of the epidermal scutes the turtle comes forth clean of algae, and bright in color. . . . During the late summer and early autumn of 1906, many small musk turtles were seen surrounded by a white halo which was conspicuous at a distance, very much resembling the general appearance of *Saprolegnia* on fishes. It was found upon examination that the white growth consisted of a dense growth of a stalked branched protozoan, *Opercularia*. Later it was found that larger musk turtles harbored considerable masses of

the protozoan on the plastron, this being frequently entirely covered, so that the turtles were practically botanical gardens above and zoological gardens below. Neither the alga nor the protozoan appears to do the turtles any injury. The algae above may assist the turtle in concealment; the protozoan below is self-supporting, feeding on minute organisms. The turtles in the muddy waters of Lost Lake are much more heavily overgrown than those of the clearer waters of Lake Maxinkuckee.

In writing of the western painted turtle, *Chrysemys marginata* Agassiz, EVERMANN and CLARK state:

As the epidermal scutes of these turtles grow old they occasionally become covered with various growths. An alga which appears to belong to the genus *Microspora* grows on the dorsal scutes, and less frequently a branched stalked *Opercularia* grows on the ventral scutes. Sometime during the year, usually in the late summer, the turtles shed these epidermal scutes, and can frequently be seen with some clean new scutes and old overgrown ones. . . . In the autumn of 1906 one of these turtles was caught with the alga on it in fruit, the base of the alga being green, while the fruiting tips had a reddish cast.

SHUFELDT (10) notes:

I remember a specimen I had that was not more than an inch in length, from the back of which grew a long tassel of elegant green moss, fully twice the length of the turtle. This moss streamed out from behind it in a very attractive fashion, as it swam the length of the aquarium.

Also, TIFFANY (11) recorded the finding of COLLINS' *Chaetomorpha chelonum* in Iowa: "Collected from backs of turtles, *Chrysemys marginata belli*, . . . taken from Miller's Bay and Lake West Okoboji, July and August, 1915 and 1923."

During the summer of 1923, while a study of the snapping turtle was being made, a specimen coated with an unusual abundance of green algae was found at St. Peter, Minnesota. It was supposed that the alga must be some common species, but to make sure a sample was collected and preserved in formalin. This specimen, when examined under the microscope, proved to be a plant with very distinctive characteristics, and easily attributable to COLLINS' *Chaetomorpha chelonum*, except for the fact that its dimensions were much larger. Its most peculiar and interesting character was not at once observable, however, and a more careful examination has indicated that this distinctive character, that of branching, justifies its receiving generic rank.

KÜTZING (7) founded the genus *Chaetomorpha* in 1845. SETCHELL

and GARDNER (9) made a very definite statement which seems to indicate that there has been no change of opinion concerning the validity of KÜTZING's original description so far as it has to do with the question of branching.

It [*Chaetomorpha*] seems to be generally recognized as a genus and to be retained in spite of the fact that it approaches *Rhizoclonium* on the one side and *Hormiscia* on the other. From *Rhizoclonium* species in typical form it is readily to be distinguished by the lack of branches of any kind, rhizoidal or otherwise.

No material of *Chaetomorpha chelonum* is available for examination, but COLLINS' (1) description proves beyond a doubt that it branches at the base at least, and that otherwise (except in size) it is in agreement with the Minnesota plant.

TIFFANY'S (11) notes on his collection of *C. chelonum* in Iowa are as follows:

Zoospores, produced throughout the first and second week of July, 1925, are very small and quite numerous in the cell, escaping through an opening near the middle of the cell. The basal cells are very long, ending in tortuous holdfast appendages. The cells gradually become shorter and thicker and the cell wall relatively thinner from base to apex of the filament. Its unique habitat makes its identification almost certain even when "on the move."

A second collection of this same plant was made in Minnesota in July, 1926, from the shell of a turtle kept in a tank of running water in the Zoological Museum, University of Minnesota. One of the basal coenocytes was measured and drawn, being 32 m $\mu$  wide and 1840 m $\mu$  long. The Minnesota specimens, except in the matter of size, agree in every respect with those of COLLINS and TIFFANY, but in addition they show the presence of horizontal rhizome-like filaments and instances of true branching in the upper portions of the erect filaments.

To examine the material under the microscope, the closely woven "membrane" must be teased apart into separate portions or small clumps of filaments having somewhat the appearance, at the base at least, of clumps of bamboo. There is no difficulty in being able to find quantities of apparently unbroken basal segments, resembling those of a true *Chaetomorpha*, detached from the membrane but having a holdfast with rhizoidal processes. On the other hand, it is almost impossible to secure a single entire plant showing the

basal branching, that is, the branching of the basal coenocyte. We were successful in a very few cases in extracting a specimen showing true branching of the basal coenocyte (fig. 2). Usually the two branches were equally strong and were of practically the same diam-

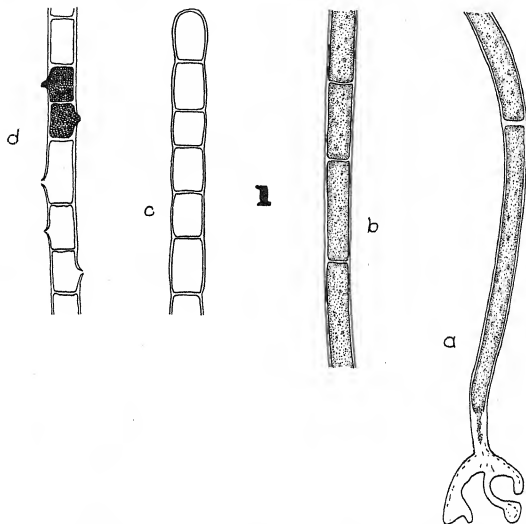
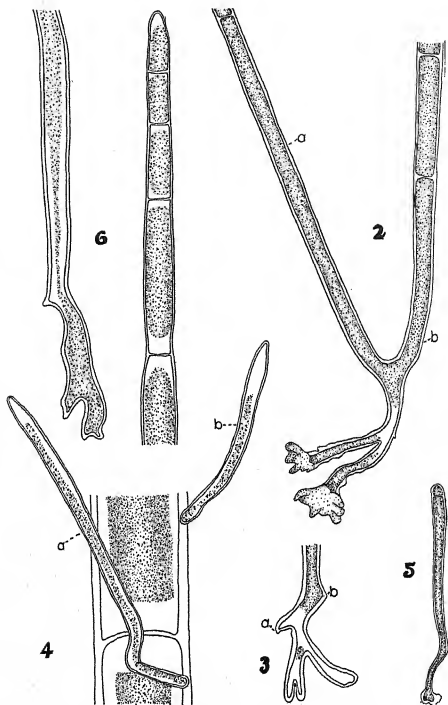


FIG. 1.—*Basicladia chelonum* (Collins) "showing the characteristically long basal cell with tortuous holdfast appendages, the gradual shortening and widening of cells from base to apex of filament, and zoospore formation." After TIFFANY.

eter and length. The basal segment of one branch (fig. 2a) was 1764  $m\mu$  in length. No good specimen of the horizontal or creeping filaments was found, but in numerous cases their presence was indicated by the broken end seen in the lower portion of basal coenocytes (figs. 3, 8).

In the presence of creeping rhizome-like branches and branched

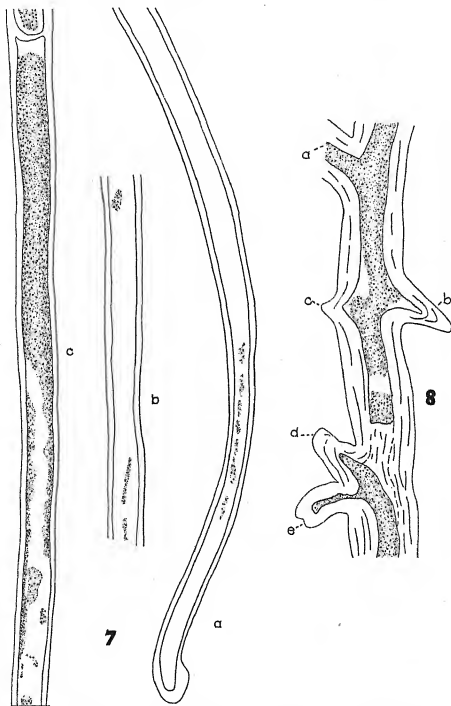


FIGS. 2-6.\*—*B. crassa*: dichotomously branched basal coenocyte of upright filament, longer member (*a*) measures  $75 \times 1764 \mu\mu$ ; shorter (*b*),  $98 \times 377 \mu\mu$ . Fig. 3, portion of holdfast showing free and coalesced rhizoidal processes and remains (?) of horizontal rhizome-like filaments (*a*, *b*). Fig. 4, two young plants attached to wall of old coenocyte (*a*,  $21 \times 262$ ; *b*,  $19 \times 182 \mu\mu$ ). Fig. 5, young plant with apical coenocyte just formed. Fig. 6, young plant consisting of six coenocytes (basal segment  $28 \times 540$ , apical coenocyte  $29 \times 75.6 \mu\mu$ );  $\times 100$ .

\* Figs. 2, 3, 5, 7, 9, 10, 13, 14, 20, 21 drawn with camera lucida by W. E. H. All other figures drawn  $\times 100$ : fig. 4, M. G. FORBERG; fig. 6, K. B. WEBB; fig. 8, W. F. ADAMS; figs. 11, 17, O. VITEK; figs. 12, 15, 16, G. BOE; figs. 18, 19, H. V. WILDES; fig. 22, H. BERGLUND.



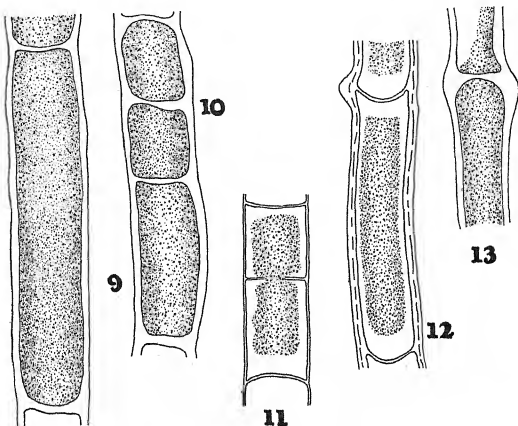
holdfasts the plant bears a close resemblance to such members of the Siphonales as *Derbesia* and *Caulerpa*. A search for branching in the upper portions of the plant was finally rewarded. A large



FIGS. 7, 8.—Fig. 7, one of longer basal coenocytes, more than 2 mm. in length; fig. 8, portion of old basal coenocyte showing points (a-e) where horizontal filaments have been broken off;  $\times 100$ .

branch was seen but was lost before a permanent mount could be made. A second and smaller specimen (fig. 22) was preserved and careful measurements and drawings have been made of it.

A cytological study of the plant is in progress and for that reason no mention is made of the cellular contents in this paper.

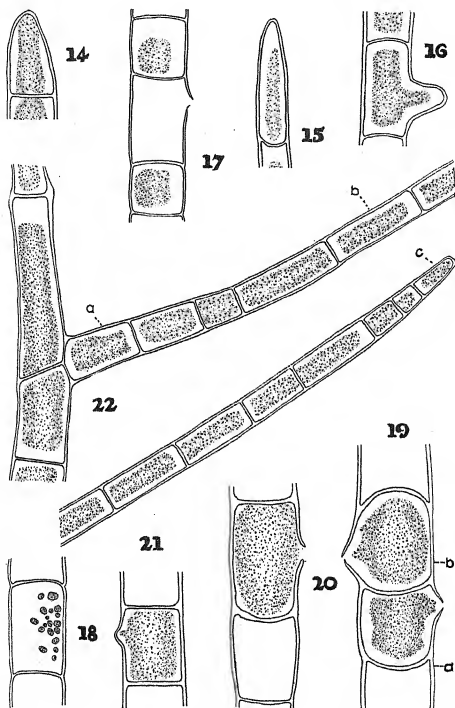


FIGS. 9-13.—Fig. 9, long coenocyte from lower portion of erect filament ( $85 \times 387 \text{ m}\mu$ ); fig. 10, two short coenocytes in median portion of erect filament; fig. 11, dividing coenocyte ( $75 \times 184 \text{ m}\mu$ ) with daughter coenocytes unequal in length; fig. 12, long coenocyte from lower portion of erect filament ( $71 \times 290 \text{ m}\mu$ ) showing wide lamellate wall thickened at node; fig. 13, coenocyte from lower portion of erect filament showing thickening of wall at node;  $\times 100$ .

The following description is prepared and the genus named.

**Basicladia** Hoffmann and Tilden, gen. nov.

Fronds consisting of numerous multicellular, erect, somewhat rigid, sparingly branched, more or less cylindrical filaments arising from creeping, rhizome-like filaments which are fastened to the substratum (shell of turtle) by holdfasts having free or coalesced branches; basal coenocytes very long, rarely branched; coenocytes



FIGS. 14-22.—Fig. 14, apical coenocyte of erect filament. Fig. 15, apical coenocyte (42  $\mu$  wide at base, 25  $\mu$  at tip, 147  $\mu$  long). Fig. 16, coenocyte in lower part of erect filament, with young branch (?). Fig. 17, empty sporangium, unchanged in shape. Fig. 18, sporangium (71 $\times$ 107  $\mu$ ) containing zoospores (gametes?) measuring 6-7 $\times$ 10-11  $\mu$ . Fig. 19, sporangia (a, 88 $\times$ 92  $\mu$ ; b, 94 $\times$ 98  $\mu$ ) with contents unchanged but pores fully developed and open. Fig. 20, sporangium showing open pore. Fig. 21, sporangium with unopened pore. Fig. 22, mature branch produced from twenty-second coenocyte from base of erect filament (basal coenocyte of branch a, 56 $\times$ 101; b, 42 $\times$ 126; c, 28 $\times$ 59.5  $\mu$ );  $\times$ 100.

gradually becoming shorter and wider from base to apex as upper coenocytes are transformed into sporangia, but immature filaments tapering in upper portions with attenuate apical coenocyte; wall of segments thick, firm, usually distinctly lamellate, sometimes thickened at nodes, relatively thinner from base to apex of upright filaments; reproduction by zoospores (?) produced in upper coenocytes, escaping through a pore.

Type species, *Basicladia crassa* Hoffmann and Tilden.

Type in the Herbarium of the Department of Botany, University of Minnesota, collected from shell of a common snapping turtle, *Chelydra serpentina*, at St. Peter, Minnesota, August 17, 1923, by William E. Hoffmann.

#### Key to species

Upright filaments reaching a diameter of 50–120  $\mu$  . . . . . 1. *B. crassa*

Upright filaments 35  $\mu$  or less in diameter. . . . . 2. *B. chelonum*

1. *Basicladia crassa* Hoffmann and Tilden, sp. nov.—Frons bright or dark green; horizontal layer composed of branching interlacing filaments from which arise upright filaments; upright filaments 2 cm. or more in height, erect, straight, somewhat rigid especially in lower portions; basal coenocytes of upright filaments 50–120  $\mu$  in diameter, up to 30 diameters or 1325–3175  $\mu$  in length; following coenocytes 70–125  $\times$  290–625  $\mu$ , 4–8 diameters long; upper coenocytes 55–73  $\times$  65–155  $\mu$ , 1–2.5 diameters long; apical coenocytes 30–95  $\times$  60–275  $\mu$ , 1.5–3 diameters long; sporangia (?) 64–127  $\times$  87–179  $\mu$ , 1–1.5 diameters long; basal coenocytes of upright filaments sometimes branched (dichotomously); upper coenocytes rarely giving off branches; upper branches long, straight, somewhat rigid, gradually tapering toward the apex, composed of many coenocytes; apical coenocyte of upright filaments and branches somewhat pointed.

Habitat: collected from shell of living snapping turtle, *Chelydra serpentina*, St. Peter, Minnesota, August 17, 1923, by William E. Hoffmann.

2. *Basicladia chelonum* (Collins) Hoffmann and Tilden, comb. nov.—*Chaetomorpha chelonum* Collins, Rhodora 9:198–200. 1907; Green Algae of North America. Tufts College Studies 2:326. 1909; Rhodora 11:196, 197. 1909. Tiffany, Trans. Amer. Micr. Soc. 45:78. pl. 12. figs. 138–141. 1926.

Basal layer a dense mass of very irregular, pluricellular, coralloid branches, forming a dense and continuous expansion on the substratum; erect filaments straight, 12-20  $m\mu$  diameter at base, increasing in size upward to 35  $m\mu$  in the upper part of the vegetative plant; lower coenocytes up to 1 mm. long, up to 50 diameters in length; following coenocytes 5-10 diameters long; upper coenocytes 2-3 diameters long; wall of coenocytes thick; sporangia in upper part of filament up to 50  $m\mu$  in diameter, 1-4 diameters long, from slightly moniliform to nearly globular; zoospores escaping by an opening near the middle of the sporangium, through a very short tube.

This species is known only from Michigan, Massachusetts, and Iowa. COLLINS' original material was found on the backs of turtles, *Chrysemys marginata* and *Aromochelys odorata*, Walnut Lake, Oakland County, Michigan, by Dr. T. L. HANKINSON. Later COLLINS made a personal collection from the shell of a turtle at Tewksbury, Massachusetts, in June, 1909. TIFFANY's specimens were collected from backs of turtles, *Chrysemys marginata belli*, taken from Miller's Bay and Lake West Okoboji, near Milford, Iowa, July and August, 1915 and 1923.

COLLINS (1) recognized the fact that the plant branches, and refers to it in no uncertain terms:

There are distinctly branching, pluricellular filaments, which unite to form a practically continuous layer on the substratum, the shell of the turtle. The basal developments of the individual plants are so closely united that the appearance is that of a continuous membrane, from which arise numerous erect, bright green, straight filaments, pretty uniformly increasing in size from the base to the apex. The cell wall is thick, in the lower cell about one quarter of the diameter; the cells in the vegetative filaments are nearly cylindrical, but the fertile cells are strongly swollen, sometimes nearly globular.

In all probability the green algae growing on the backs of turtles mentioned by the various writers quoted in this paper belong either to one or to the other of the two species described. It is hoped that other collectors may discover new material and give it further study.

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## LITERATURE CITED

1. COLLINS, F. S., Some new green algae. *Rhodora* 9:198-200. 1907.
2. ———, The green algae of North America. *Tufts College Studies* 2:326. 1909.
3. ———, An algological prophecy fulfilled. *Rhodora* 11:196-197. 1909.
4. DITMARS, R. L., The reptile book. p. 14. 1915.
5. EVERMANN, B. W., and CLARK, H. W., The turtles and batrachians of the Lake Maxinkuckee region. *Proc. Ind. Acad. Sci.* 1916. 477-493. 1916.
6. GADOW, H., Amphibia and reptiles. p. 340. 1901.
7. KÜTZING, F. T., *Phycologia germanica*. p. 203. 1845.
8. POTTER, M. C., Note on an alga (*Dermatophyton radicans* Peter) growing on the European tortoise. *Jour. Linn. Soc. Bot.* 24:251-254. 1887.
9. SETCHELL, W. A., and GARDNER, N. L., The marine algae of the Pacific coast of North America. *Univ. Calif. Publ. Bot.* 8:198. 1920.
10. SHUFELDT, R. W., Observations on the Chelonians of North America. *VII. Aquatic Life* 16: February. 1920.
11. TIFFANY, L. H., The filamentous algae of northwestern Iowa with special reference to the Oedogoniaceae. *Trans. Amer. Micr. Soc.* 45:78. 1926.

# FERTILIZATION IN A LIVING OEDOGONIUM

EARLE AUGUSTUS SPESSARD

(WITH ELEVEN FIGURES)

## Introduction

The genus *Oedogonium* is so large, so well investigated, and so widely used that new observations on the process of fertilization in any of its species must be of interest. No doubt many investigators have actually observed the act of fertilization in *Oedogonium*; not the cytological aspects as shown by fixed and sectioned material, but the passage of a living sperm into a living egg.

Such taxonomic works as those of HEERING (1) and HIRN (2) would not be expected to devote space to this matter. KLEBS (3) was more interested in the control of fertilization than in a description of the act. OLTMANN (6) treats the matter in a comparative way from the morphological standpoint, as do also WEST and FRITSCH (8). KLEBAHN (4) has furnished a widely used illustration of fertilization in *O. boscii*. This figure, as reproduced in ENGLER and PRANTL, shows a sperm with the cilia pointed away from the egg, a condition which certainly never occurs at fertilization in the species investigated by the writer. Miss OHASHI (5) has observed fertilization in *O. nebraskense*.

This account deals only with the time element in the formation of sex organs, their period of maturing, the length of life of sperms, the time consumed in the act of fertilization, and the destiny of unsuccessful sperms. A short account is given also of the sperm.

## Material

The plants of *Oedogonium* used were secured from a running stream in Clark County, Arkansas, November 3, 1928. No sex organs were visible when the plants were collected. The material was placed in culture and on November 10 separated into three masses. Two were placed in 20 cc. of river water in 25 cc. collecting vials, stoppered, and set in an east window. The third was used to distribute among five current cultures of various other algae. *Hydra*

*viridis* was placed in the two vials to keep under control the excessive reproduction of small crustaceans, which are harmful to many filamentous algae. *H. fusca* will do just as well for this purpose provided there is not an excess of sunlight and provided the container is spacious and uncorked. These two vials furnished enough material\* to observe all the stages in sex reproduction and zoospore formation within thirty days. Besides this material, they also furnished enough to begin new cultures. It is hoped that a means for growing the plant in pure culture may be found, so that its food requirements may be studied as well as the factors controlling reproduction. It is only through single zoospore isolation cultures that the life of a plant can be established.

The species under investigation has been identified by Professor L. H. TIFFANY as *Oedogonium kurzii* Zeller. I wish to thank him for his cooperation in the solution of this part of the problem.

#### TIME AND SEX ORGAN FORMATION

In vial *A* the first oogonia were seen November 15. These were almost ripe for fertilization and the antheridia were discharging in the afternoon of the following day. This set of sex organs was used for preliminary observations. Vial *B* furnished a rather complete record. The plants in it continued to vegetate until November 24, when the first oogonia were seen. The next day antheridia began to appear. By November 27 both were mature, and the next morning fertilization was observed to occur twice (table I). Fertilization had occurred in many oogonia in this vial previous to the observation of fertilization, as indicated by the number of sperms caught and observed to die within the oogonium. From the records of individual filaments it appears that fertilization may occur within forty-eight hours after the sex organs begin to differentiate.

#### PERIODICITY

There is a distinct periodicity observable in the production of sex organs. From the limited observations made on this point, there is some evidence that sex organs are formed at high peaks every fifteen days. A few are formed during the interval, but for the most part this time is consumed in a vegetative activity. The filaments finally become completely transformed into reproductive cells alternating with vegetative ones. The latter die and the filament fragments.



The antheridia of one filament develop a day later than the oogonia of the same filament. This insures against self-fertilization.

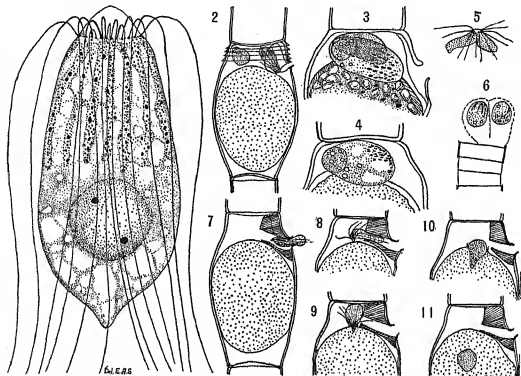
The most conspicuous periodicity is seen in the discharge of the sperms. They discharge in swarms between midnight and 4:00 A.M. There is a smaller display in the early hours of the afternoon. It is certain that they are discharged at any hour of the day, but to one who observes them continuously for a 24-hour stretch, this sudden increase in discharge of sperms is very evident. The oogonia, alternating with the antheridia, also show this periodicity.

#### LIFE OF A SPERM

While the life of a filament from the time it begins to produce sex organs until it is exhausted is but a few days, the life of a sperm can be counted in hours. Table I shows that no sperms of this species lived more than thirteen hours, and that most of them succumbed before that time. Many sperms were seen to enter the oogonium and were timed for the death point. Generally they ceased to move within two hours and then slowly enlarged to as much as twice their size and disintegrated about four hours later. This fact causes great loss of time to the investigator. The fact is that if he does not see fertilization take place at once, the sperm under observation will prove to be a dying one.

It is this point that differs from an interpretation of KLEBAHN's figure. Invariably a sperm entering an oogonium in which the egg is already fertilized enters with the same avidity which sperms exhibit entering one not fertilized. Unlike the latter, they have not been observed to turn toward the egg, but on the contrary away from it, so that they come to rest or rotate with the ciliated end pointed into the farthest corner away from and above the egg, precisely as shown in KLEBAHN's figure. In other words, a sperm in that position does not represent fertilization but a sperm that has entered an oogonium, the egg of which has already been fertilized. This criticism of KLEBAHN's figure is not a reflection upon his observations, but calls attention to the unfortunate labeling of a preparation that is in the opinion of the writer slightly misleading. Figs. 2-4 show the sperm during its life in an oogonium in which the egg has already been fertilized. It is not rare to find two sperms in such an oogonium, and three were observed once.

The sperm is subject to the ordinary vicissitudes of its environment during its short life. Numbers 2 and 11 in table I illustrate what can and does frequently happen to swimming sperms. Destruction by foraging animals has been observed five times in all. Sperm number 9 illustrates the point that eviction of the sperm within a short time after discharge is necessary. A number of sperms were



FIGS. 1-11.—Fig. 1, sperm of *Oedogonium kurzii* showing large nucleus in colorless posterior region and chromatophore of anterior region; smaller vacuoles on either side of nucleus are contractile (dorsal view);  $\times 1925$ . Fig. 2, oögonium containing fertilized egg and two sperms;  $\times 192$ . Figs. 3, 4, upper regions of oögonia with sperms enlarged and disintegrating; small circles are contractile vacuoles, dark splotches coloring matter;  $\times 352$ . Fig. 5, deformed sperm which reached mouth of oögonium but could not enter;  $\times 192$ . Fig. 6, discharge of two sperms from antheridium;  $\times 192$ . Figs. 7-10, passage of sperm through oögonial conduit into fertilization chamber, establishing contact (fig. 8) and entering egg thirty seconds later (figs. 9, 10);  $\times 192$ . Fig. 11, position of sperm two hours after entering egg;  $\times 192$ .

observed in this and another species to be discharged not simultaneously. This may or may not be due to a faulty mechanism, but in every instance such sperms experienced difficulty in escaping from the membrane after discharge from the antheridium. Several of these were entirely unable to escape and died within the sheath after the normal manner.

One sperm (fig. 5) was deformed. It swam about for an hour and found an oogonium. The opening was not large enough to accommodate the deformity and the sperm died in due time at the pore.

Table I lists the history of the lives of fifteen sperms which were under constant observation during the periods indicated. A larger number of sperms were observed for various periods of time, but these are not listed because they are not complete records.

TABLE I  
HISTORY OF FIFTEEN SPERMS OF OEDOGONIUM KURZII

| INDIVIDUAL NUMBER | HOURLY<br>DISCHARGED | HOURLY<br>ENTERED | HOURLY<br>MOVEMENT<br>CEASED | HOURLY<br>SPERM<br>DISINTEGRATED |
|-------------------|----------------------|-------------------|------------------------------|----------------------------------|
| 1. ....           | 2:00 P.M.            | 3:00 P.M.         | 10:18 P.M.                   | 12:01 A.M.                       |
| 2. ....           | 2:00 P.M.            | Never             | Devoured                     | 11:30 P.M.                       |
| 3. ....           | 2:00 P.M.            | 3:00 P.M.         | 11:20 P.M.                   | 2:50 A.M.                        |
| 4. ....           | 10:00 A.M.           | 11:00 A.M.        | 1:05 P.M.                    | 1:20 P.M.                        |
| 5. ....           | 2:00 P.M.            | 2:30 P.M.         | 5:10 P.M.                    | 7:00 P.M.                        |
| 6. ....           | 8:15 A.M.            | 9:43 A.M.         | 10:30 A.M.                   | 2:00 P.M.                        |
| 7. ....           | 6:00 P.M.            | 11:00 P.M.        | 11:50 P.M.                   | 4:00 A.M.                        |
| 8. ....           | 3:00 P.M.            | 4:30 P.M.         | 6:30 P.M.                    | 12:00 M.                         |
| 9. ....           | 3:00 P.M.            | Never             | 5:15 P.M.                    | 11:00 P.M.                       |
| 10. ....          | 2:35 P.M.            | 2:45 P.M.         | 8:45 P.M.                    | 1:30 A.M.                        |
| 11. ....          | 10:00 A.M.           | Never             | Devoured                     | 10:03 A.M.                       |
| 12. ....          | 9:35 P.M.            | 9:40 P.M.         | 12:45 A.M.                   | 2:35 A.M.                        |
| 13. ....          | 3:40 P.M.            | Never             | 5:20 P.M.                    | 1:20 A.M.                        |
| 14. ....          | 2:00 A.M.            | 2:05 A.M.         | Fertilization at 2:06 A.M.   |                                  |
| 15. ....          | 2:15 A.M.            | 2:45 A.M.         | Fertilization at 2:49 A.M.   |                                  |

Sperm number 1 swam out of the oogonium at 9:00 P.M. and number 5 at 5:00 P.M. These two sperms died on the slide. Number 9 failed to escape from the sheath after discharge from the antheridium.

#### TIME ELEMENT IN FERTILIZATION

The next question is naturally concerned with the act of fertilization itself, or rather that phase of it taken up by the passage of the sperm into the egg. This act was observed twice, in sperms 14 and 15 of table I. It is obvious from the table that considerable time may be spent in following false trails. This is due to the absence of knowledge regarding the most likely time for the event to occur, a knowledge of the periodicity involved, and, what is most important, a knowledge of the time it actually takes for a sperm to enter the egg. It is apparent from the table that if one is going to follow the method of following the trail of a sperm from the time it leaves the antheridium until it enters the egg, the chances are much in favor of

disappointment. It is interesting to observe the slow changes of a disintegrating sperm; but it is also conducive to erroneous interpretations if one is ignorant of the fact that the sperm is actually disintegrating and not entering the egg by a slow process. The nucleus becomes amoeboid during the last phases before death; the contractile vacuoles become very active and may number as high as sixteen. The sperm will increase to twice its original size and the nucleus will come to lie immediately in contact with what appears to be the egg membrane. In several cases fine protoplasmic lines apparently were seen to connect the nucleus and the egg. In another the starch grains of the egg were seen to move over the nucleus after the cytoplasm had become highly vacuolated, so that the nucleus appeared to be three-fourths of the way into the egg. During the time it takes to slip the camera lucida prism into place, perhaps thirty seconds, the protoplasm had burst and the nucleus had disappeared. This seemed to be genuinely a case of slow passage of the nucleus into the egg, leaving the balance of the sperm in the oogonium. But since the actual destiny of the nucleus was lost by thirty seconds of necessary diversion after a continuous observation of some nine hours, it had to be discarded as worthless data. Passage of the sperm into the egg is not a slow process.

On November 28, twenty-four days after the plants were brought from the field, and two days after the appearance of sex organs, two sperms were observed to enter separate eggs. The first consumed thirty seconds passing through the conduit into the fertilization chamber, and one second getting into position to enter the egg or making contact with it. The actual passage into the egg took thirty seconds. Judging from the length of the sperm, it traveled in this passage at the rate of 1.5 mm. per hour. The movement was a gliding one. The sperm did not change its shape during the passage although its lower mid-region was larger than the forward end. The most startling feature of this passage was the behavior of the egg. The cytoplasm spread apart in a slow splash so that it moved upward around the sperm with the fringes of the opening bent away from it. There was no sudden quickening of the movement of the sperm after the thickest portion had entered. As the tail end of the sperm disappeared into the egg substance this healed over, leaving no trace of the sperm's passage.

The second case differed from the first in no important particular. It consumed slightly more than four minutes getting through the conduit. In this passage the inner opening was smaller than the diameter of the sperm, which showed an undulating body movement similar to that of a *Paramoecium* passing through a hole. Its normal shape was resumed when inside the fertilization chamber. This sperm was seen to have a one-sided snout with which it made contact with the egg as soon as the head end had passed the inner portal of the conduit. There was no hesitation on the part of the sperm to assume a position vertical to the egg and establish its contact. Once this contact was made the sperm glided into the egg precisely as described for the other one. There was a distinct hyaline and granular fringe of cytoplasm observable in the egg, however, as it opened to receive the sperm, or rather as the sperm pushed the egg apart. This may be the receptive spot. To say that the egg opens for the sperm or that the sperm pushes open the egg is not accurate. There is no movement of the cilia which lie backward along the sperm that can account for the passage. More cases will have to be observed to explain the forces which cause the two organisms to function. This sperm could be seen within the egg after entry. It stopped in the upper part and remained there for two hours, at which time observation of it ceased.

It is clear from these observations of two sperms that the actual passage of the sperm is phenomenally rapid, taking about half a minute. The passage through the conduit may perhaps require five minutes, but this is probably unusual judging from observations made on numerous other sperms as they entered oogonia. The sperm generally manages to pass through the conduit in less than a minute. Actually then, fertilization normally occurs within a minute after the sperm reaches the oogonium. It sometimes reaches the oogonium within a minute after its discharge from the antheridium, so that it is possible that the entire process from sperm discharge to passage into the egg may transpire within two minutes. This means that the observer has little chance of seeing the process. If the period of maximum discharge of sperms is known, however, the observation is not difficult to make. It is certainly unprofitable to follow the sperm. If a sperm does not enter the egg at once after entering the oogonium, the egg of that organ may be assumed to have been fertilized previously.

## LIVING SPERM

All the writers referred to in this paper seem to concur in the opinion that the sperm of *Oedogonium* is a miniature zoospore in appearance. The sperm of this species is larger than the zoospore of most species of the genus, so that its movements may easily be seen. A few points will be mentioned to show that this opinion of the nature of the sperm must be thoroughly examined.

The sperm of this species shows indications of dorsiventrality in several respects. First, at least one was observed to possess a definite apical point at the moment of contact with the egg. This point did not include the entire apical termination of the sperm but only about half of it. It was impossible to determine if the point was dorsal or ventral in position. In all specimens killed for observation the point was not observable, but killing the sperm alters its shape profoundly. The cilia which are normally extended backward along the body, unless in actual use, are slowly bent forward and become much shortened. This is the position shown in most figures; therefore any observations made on fixed material of sperms must be checked with observations of living sperms in this species at least. Second, the movement of the organism is more like that of a dorsiventral animal than one having another type of symmetry. The animal possesses cilia that are apparently equal in length although they seem not to be used in a wavelike rhythm. They are used more like oars, with possibly a few extending forward permanently as tactile organs. This is a degree of specialization not observed in zoospores, and can be explained on the basis of dorsiventrality.

Besides apparent dorsiventrality, the oscillating movement of the sperm is very unlike the rotating movement of zoospores of this and other species of *Oedogonium*. Also, the coloring matter of the sperm, admitting its evident reduction, is definitely distributed in the more anterior region of the body. There is no starch in the sperm, although this feature is undoubtedly connected with the reduced amount of chlorophyll. However, sperms and zoospores come from the same sort of cells within a few days of vegetative activity. The presence of oil in one and the absence of starch in the other indicate at least a chemical specialization or adaptation in the sperm not present in the zoospore. There are contractile vacuoles in the sperm

of this species. This organ has not yet been seen in the zoospores of the same species.

In the species under investigation, the sperm seems not to be a miniature zoospore but a highly differentiated cell differing from zoospores in important particulars.

### Summary

1. Sex organs are mature approximately forty-eight hours after they begin to form.
2. The antheridia appear about a day after the oogonia, insuring cross fertilization between filaments.
3. The high peak in sex organ production occurs about every fifteen days.
4. The antheridia open and sperms are discharged most frequently between midnight and 4:00 A.M. Between noon and 4:00 P.M. there is another copious discharge.
5. The unsuccessful sperm lives between two and thirteen hours.
6. Fertilization may occur within two minutes after discharge of the sperms.
7. Passage of the sperm into the egg takes thirty seconds.
8. The sperm is not to be considered a miniature zoospore.

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### LITERATURE CITED

1. HEERING, W., in PASCHER'S Suesswasserflora. Heft 6, Band 3. 156-244. 1914.
2. HIRN, K., Monographie und Iconographie der Oedogoniaceen. Helsingfors. 1900.
3. KLEBS, G., Fortpflanzung bei Algen und Pilzen. Jena. 1896.
4. KLEBAHN, H., Die Befruchtung von *Oedogonium boscii*. Pringsh. Jahrb. 24:244. 1892.
5. OHASHI, HIRO, *Oedogonium nebrascense* sp. nov. BOT. GAZ. 82:207-214. 1926.
6. OLTMANN, F., Morphologie und Biologie der Algen. 1:330-342. 1922.
7. PRINZ, H., Chlorophyceae in ENGLER and PRANTL, Die natürlichen Pflanzenfamilien. 3:244-252. 1927.
8. WEST, G. S., and FRITSCH, F. E., A treatise on the British freshwater algae. Cambridge. 212-224. 1927.

# CYTOLOGICAL FEATURES OF THE LIFE HISTORY OF GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 404

EDITH STEVENS

(WITH PLATES VII, VIII)

## Introduction

The aim of the investigation here reported was to determine some of the general cytological features in the life history of *Gymnosporangium juniperi-virginianae*. The first intention was to work in detail the entire history, but the new questions which arose as the investigation progressed and the limited time did not permit such an extended study. The investigation deals with the origin of the teliospore, its ripening, germination, the reduction divisions, formation of the basidiospore, and finally includes a preliminary account of the aecia.

## Material and methods

Cedar galls or "cedar apples" of *Juniperus virginiana* were collected in Indiana, Virginia, West Virginia, and Tennessee. Collections were made at weekly intervals during March, April, and May. Infected leaves were obtained from inoculations made by Dr. MOTTIER of Indiana State University, who collected twigs infested with *Gymnosporangium juniperi-virginianae* and placed them over *Pyrus coronaria* leaves in order to be certain of procuring infection. Inoculations of apple seedlings with *Gymnosporangium* were made in the University of Chicago greenhouse during the month of March, and in this manner stages in the development of pycnia and aecia were obtained. The next step was to inoculate *J. virginiana* with the aeciospores, then the complete life history of one species of *Gymnosporangium* would be assured. The species used was identified by careful checking of morphological differences of the teliosori, teliospores, the gall, and the host plants. By the process of elimination the species could be no other than *Gymnosporangium juniperi-virginianae*.



Most of the material for this investigation was killed in formalin-acetic-alcohol and stained with iron-haematoxylin. The galls were cut in tiny squares, each containing a single teliosorus, in order that the killing might occur as quickly as possible. The teliospore horns were detached from the gall, run through a close series of alcohols into glycerin, the glycerin washed out with absolute alcohol, and then taken through a close series of cedar oil (diluted with alcohol) into pure cedar oil. The material brought into paraffin by this method was easily cut at any desired thickness, but if the gelatinized horns were carried through the usual series of alcohols and xylols, the material became flinty and impossible to cut. If the horns were killed in chromo-acetic solution the gelatin disappeared, leaving only the teliospores, which were placed in onion skin bags and carried through the alcohols and xylols into paraffin in the usual manner, and were easily sectioned. Most of the sections were 5 or  $7\mu$ ; if cut less than  $5\mu$  the sections were so fragmentary that it was hard to obtain satisfactory conclusions from them.

Germinating teliospores were stained and transferred to glycerin and Venetian turpentine, and the whole mounts which were made proved to be very satisfactory.

In order to obtain a large quantity of infected apple leaf material, seeds from Jonathan apples were planted February 13, 1928, and in the beginning of March inoculations of the seedlings were begun. Cedar apples placed in a very moist chamber were allowed to stand overnight. By that time the teliospores had sent out basidia and the basidiospores had formed, the latter easily discerned with the naked eye by the white appearance of the horns. The apple leaves were rubbed lightly to rid them of bloom, sprayed with distilled water, and the spores, suspended in water, dropped upon them. The plants were immediately placed in a glass cage and allowed to incubate for two days, when they were removed to the greenhouse atmosphere. The amber-colored pycnia appeared within eleven days and mature aecia within sixty days. If the leaves were not first rubbed and sprayed with water, however, the results were not so satisfactory, and often the leaves remained as healthy as before the treatment.

### Investigation

#### ORIGIN OF TELIOSPORE

The cedar gall is made up of parenchymatous tissue, abundant mycelial threads of the fungus appearing everywhere, ramifying throughout the gall between the cells of the host. Immediately below the surface of the gall, where the teliospores are to be formed, there arise from the ends of the mycelial threads rows of parenchyma-like cells which are binucleate, like the mycelium from which they come (figs. 1-17). At the beginning of this process the ends of the mycelial threads orient themselves almost at right angles to the surface of the gall (figs. 3-8); the end cells divide vegetatively, the two nuclei in each cell dividing simultaneously and conjugately. Each nucleus elongates, and a split in the chromatin material suggests the presence of two chromosomes; while at the end of each nucleus may often be observed a dense area resembling a centrosome. Each nucleus becomes more and more elongated until it is practically pulled into two portions, the two parts being held together by a delicate strand which eventually disappears. Each of the four portions of chromatin round off into a nucleus (fig. 12), each cell containing two nuclei similar to those of the mother cell. This process has formed two cells, and the end cell then quickly forms two cells, making a row of three parenchyma-like cells (figs. 14, 15) as already mentioned. The upper cell gradually swells, becomes turgid, and loses its cytoplasm while the nuclei disintegrate (fig. 15). These upper turgid (buffer) cells help to rupture the corky layer covering them. The true basal cells, those immediately below the buffer cells, become active, and grow into and finally through the latter. There may be more than one outgrowth, often two (fig. 24), from a basal cell. While the basal cell is lengthening its nuclei divide conjugately, and one pair moves into each outgrowth or bud where they divide (fig. 23), one of the resulting pairs moving to the upper end of the cell. A wall then forms between the pairs of nuclei, cutting off a stalk cell from a terminal one (fig. 26). In this end cell the nuclei enlarge to normal size; then nuclear division occurs, giving rise to a four-nucleate teliospore mother cell (fig. 29). A wall is soon laid down, dividing the mother cell into two daughter cells which are the two cells of the teliospore, each containing two nuclei (fig. 30).

During the period of ripening the walls of the teliospore cells become much thickened, and the two nuclei in each of its cells unite, initiating a uninucleate condition (figs. 31, 32). The fusion nuclei in the teliospore show various phases (figs. 33-39), the final resting condition being shown in fig. 38.

#### TELIOspore GERMINATION AND FORMATION OF BASIDIOSPORE

About the first of March the teliospore germinates readily; earlier than this the spores are not fully ripened and will not respond to conditions which are favorable for germination later in the season. The first indication of germination is the formation of papillae, globular protrusions of the cell contents through the germ pores; but since the single nucleus of a teliospore cell rarely divides within the cell, only one protrusion can develop into a basidium. As the protoplasm of the cell passes out the mass gradually elongates, forming the basidium, while the nucleus lengthens and passes to the middle of the filament. In several preparations the nucleus, as it passed through the germ pore, was observed to be much elongated and showed two distinct thickened threadlike masses (fig. 40). This process was assumed to be a precocious division of the nucleus, the assumption being borne out by several cases in which a nucleus was found in the germ tube and at the same time one still remained within the teliospore cell.

After the fusion nucleus has passed into the basidium, the chromatin appears on one side during synapsis, the nuclear phase initiating the reduction division. The nuclear membrane disappears and the chromatin begins to elongate into two dumb-bell-shaped masses, for the two portions of the fusion nucleus string out separately and the chromatin of each shows a faint split, indicating four chromosomes, the diploid number (figs. 46, 47). The short spindle is formed and gradually increases in length, while the chromatin moves toward the poles in the form of the two elongated masses, each consisting of four chromosomes. As the division draws to a close the nuclei are formed, each containing four chromosomes; a cross wall is immediately formed and the two new nuclei quickly divide. This time there is no splitting, two of the four chromosomes in each nucleus going to one pole and two to the other, so that four nuclei are formed, each with two chromosomes (the haploid number), indicating that reduction

has occurred. Transverse walls are laid down and each of the four cells of the basidium develops a tube, broad at the base and tapering to the top, the sterigma, which becomes inflated at the tip to form a basidiospore. A sterigma is formed by each of the four cells of the basidium, probably due to a weakened place in the cell wall; but why it is always located at the same point on the cell is not understood. The wall may be weakened by enzymatic action, and the pushing out would then result from the turgidity of the cell which is to produce the sterigma. Upon the formation of the sterigma the nucleus is either pulled or pushed into it. It may be that the nucleus is attached to a centrosome by a contractile strand which pulls it into the basidiospore, as was found to be the case in *Nidularia*. Often a precocious division of the basidiospore nucleus takes place, producing a spore containing two nuclei (fig. 56); for the spores immediately germinate. In some cases the four cells of the basidium put out longer germ tubes and do not form basidiospores. Basidiospores were seen which upon germination formed secondary spores.

#### PYCNIA AND AECIA

About eleven days after inoculation, pycnia appeared as small amber spots on the upper surface of the leaf, developing between the epidermis and palisade layer. The mycelium gathers at a point just under the epidermis, and as it increases pressure is exerted on the tissue above, pushing it upward. The mycelium resolves itself into a sunken spherical structure whose periphery is surrounded by paraphyses, which later rupture the epidermis of the leaf.

As the aecia develop there is a proliferation of the cells of the leaf, and they sink into the host tissues as far as the palisade cells. It is known that the binucleate aeciospores are cut from a fusion cell, formed by the union of two uninucleate hyphae. The two cells which fuse have been thought to be similar, but it does seem that there might be a difference in them. It may be possible that a mycelium like that in the pycnia unites with that of the aecia, for the aecia rise in the neighborhood of the pycnia and do not form unless pycnia are first formed.

#### Discussion

This study of *Gymnosporangium juniperi-virginianae* agrees in the majority of the details, except in the reduction division, with other

findings in the same genus. In *G. clavariiforme* BLACKMAN<sup>1</sup> does not agree with SAPPIN-TROUFFY<sup>2</sup> that in the reduction division the chromatin masses are chromosomes. In the present investigation the split in the chromatin material would indicate that SAPPIN-TROUFFY was correct in his assumption.

Often two small nuclei are found in a teliospore cell instead of a single large one. BLACKMAN states:

These are, no doubt, the original paired nuclei of the teleutospore, which for some reason have delayed their fusion. Their fate is unknown, as two nuclei were never observed to pass into the germ-tube; probably they fuse later. There was no evidence that they represented the results of a precocious division.

The present work indicates that the late appearance of two paired nuclei within the teliospore cell may be the result of a precocious division, since frequently the fusion nucleus was observed to be in a process of division as it passed into the basidium (fig. 40).

The basidiospores are frequently binucleate, but this, like the two nuclei in the teliospore, is a precocious division in which the formation of a wall has been delayed.

### Summary

1. A row of three parenchyma-like cells arise from the end of a mycelial thread, the upper one being the buffer cell and the one immediately below it the basal cell.
2. The cytoplasm of the buffer cell disintegrates, and buds of the basal cell grow up into and through the buffer cell. The bud cell divides and forms two, the stalk cell and the teliospore mother cell.
3. The nuclei of the teliospore mother cell divide, giving rise to a four-nucleate cell. A wall is laid down dividing the cell into two daughter cells each containing two nuclei.
4. The two nuclei in each of the teliospore cells unite, initiating a uninucleate condition.
5. The walls of the teliospores in the outer portion of the teliosorus are much thicker than those toward the center.
6. Upon ripening each cell of the teliospore sends out a basidium

<sup>1</sup> BLACKMAN, V. H., On the fertilization, alternation of generations and general cytology of the Uredineae. *Ann. Botany* 18:323-375. 1904.

<sup>2</sup> SAPPIN-TROUFFY, P., Recherches histologiques sur la famille des Uredinées. *Le Botaniste*. 5<sup>e</sup> serie. 59. 1896.

where reduction takes place, four cells being formed as a result of the process. The haploid number of chromosomes is two, and the diploid four.

7. Each of the four cells of the basidium forms a sterigma, which rounds off to form a basidiospore, the nucleus of each of the cells moving up through the sterigma into the spore.

8. Basidiospores often contain two nuclei as a result of a precocious division and the delay of the formation of a wall.

Grateful acknowledgment is made to Professor CHARLES J. CHAMBERLAIN, under whose direction this investigation has been conducted.

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#### EXPLANATION OF PLATES VII, VIII

FIGS. 1, 2.—End of mycelial thread in process of division.

FIGS. 3.—Group of parenchyma-like cells.

FIGS. 4-9.—End of mycelium in process of division.

FIGS. 9-12.—First cell near completion of division.

FIG. 13.—Cell in process of division, forming two cells (basal and buffer).

FIG. 14.—Three cells in row; top is buffer cell, one just beneath is basal cell.

FIG. 15.—Nuclei and cytoplasm of buffer cell disintegrating.

FIG. 16.—Buffer cell empty.

FIG. 17.—Basal cell with four nuclei; slight bulge on basal cell shows position of outgrowth arising from it.

FIGS. 18-20.—Basal cell with bud cell cut from it and growing into buffer cell.

FIG. 21.—Cell which originated from basal cell, exerting pressure inside walls of buffer cell.

FIG. 22.—Buffer cell wall broken by bud cell (with four nuclei); cell will form stalk and teliospore mother cell.

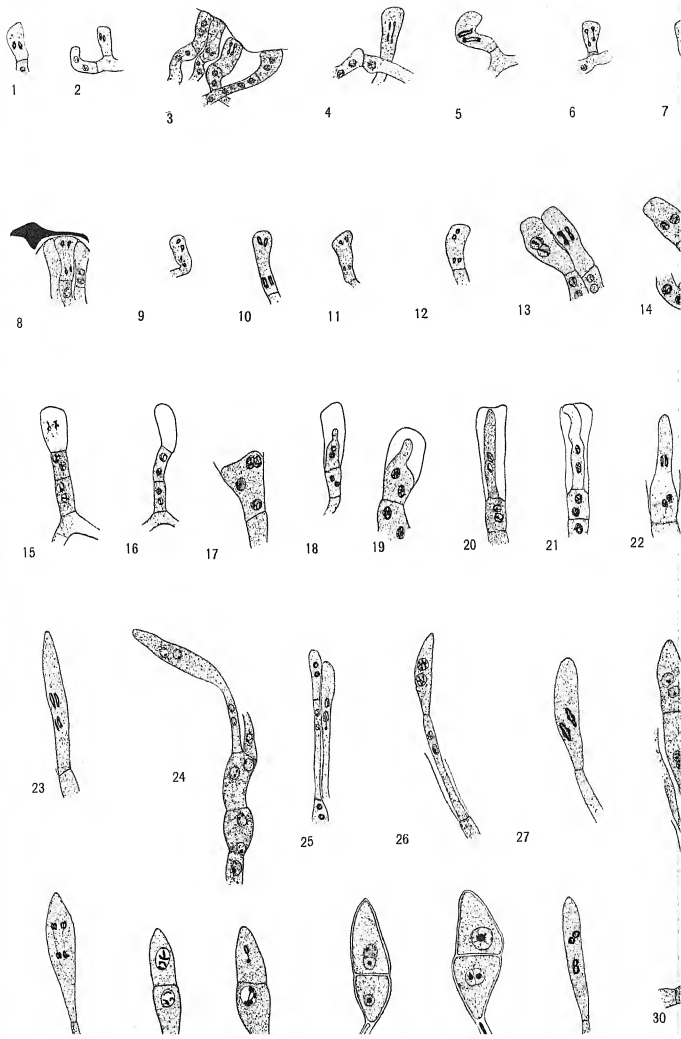
FIG. 23.—Nuclei of bud cell in process of division.

FIG. 24.—Four-nucleate bud cell which will form stalk and teliospore mother cell.

FIGS. 25, 26.—Stalk cell cut off and teliospore mother cell formed.

FIGS. 27, 28.—Binucleate teliospore mother cell dividing to form two cells of teliospore.

FIG. 29.—Teliospore mother cell with nuclear division completed.







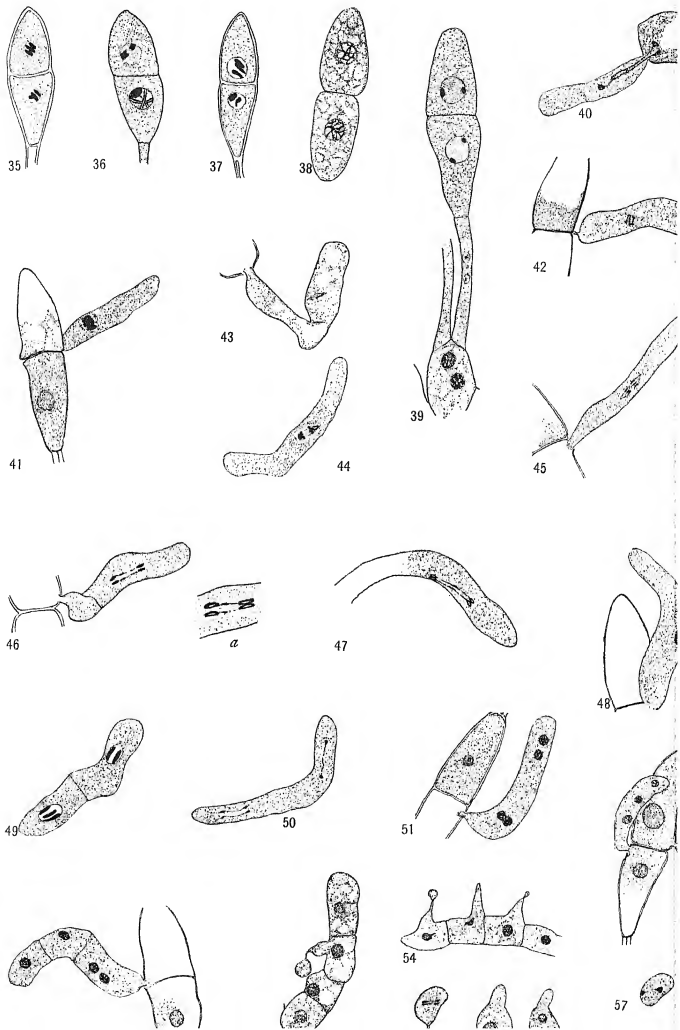




FIG. 30.—Teliospore cells formed, each containing two nuclei.

FIGS. 31-33.—Nuclear fusion in cells of teliospore.

FIGS. 33-38.—Nuclear fusion completed, chromosomes still evident.

FIG. 38.—Teliospore with resting nuclei.

FIG. 39.—Basal cell showing two outgrowths, one having formed stalk cell and teliospore.

FIG. 40.—Germinating teliospore showing precocious nuclear division.

FIGS. 41-43.—Nuclear phases in basidium.

FIGS. 43-46.—First nuclear division in basidium, showing four chromosomes moving to each pole.

FIGS. 46-48.—First division of nucleus in basidium; fig. 46a, detail of division showing split denoting diploid number of chromosomes.

FIG. 48.—First nuclear division in basidium completed; wall not formed.

FIG. 49.—First wall formed in basidium.

FIG. 50.—Second nuclear division in basidium, where reduction from four to two chromosomes takes place.

FIG. 51.—Basidium with four nuclei; walls not yet formed.

FIG. 52.—Teliospore showing basidium containing three nuclei; one division must have taken place within teliospore cell and one nucleus has not moved into basidium.

FIG. 53.—Basidium with three cells; one wall late in forming.

FIG. 54.—Basidium with sterigmata, two showing basidiospore in process of formation.

FIG. 55.—Basidium with four cells.

FIGS. 56, 57.—Basidiospores showing precocious division of nuclei.

FIG. 58.—Uninucleate basidiospore in process of germination.

FIG. 59.—Binucleate basidiospore in process of germination.

## CYTOLOGICAL STUDIES IN THE BETULACEAE

### III. PARTHENOGENESIS AND POLYEMBRYONY IN *ALNUS RUGOSA*

ROBERT H. WOODWORTH

(WITH PLATE IX AND TWO FIGURES)

#### Introduction

While making collections of staminate catkins of *Alnus rugosa* (Du Roi) Spreng. for the study of microsporogenesis, certain observations seemed significant. The plant was noted to be highly polymorphic. Extensive tracts were found to bear very few of the pollen-bearing aments. Investigation showed the microsporogenesis to be typical of heterozygous plants whose parents do not have close affinities (6). The reduction division in all phases is entirely irregular, resulting in wholesale malformation of the pollen, only 2 or 3 per cent of which appears to be morphologically perfect. Practically every plant in acres of ground cover bore the fertile catkins in great abundance. That the seeds are viable is shown by the great number of seedlings which grow about the mature plants. It was deemed of interest to learn how such great numbers of seeds are formed when the staminate catkins are so scarce, and productive of practically no viable pollen grains.

#### Material and methods

Pistillate catkins were collected weekly from the time of pollination in early spring until mid-August. Carnoy's fluid was used for killing and fixing. The aments were carefully picked apart and the tiny ovaries placed on small squares of cardboard, about 100 on a square, according to JEFFREY's mass method (3). The stain used was Haidenhain's iron haematoxylin. Drawings were outlined with a camera lucida and photomicrographs made with a Bausch and Lomb model K photomicrographic camera and a Zeiss microscope equipped with apochromatic lenses, compensated oculars, and a NA 1.40 Abbe condenser.

### Embryo sac formation

Although the pollen is shed in early spring the embryo sac mother cell does not appear until some 3 months later. SWINGLE (5) writes of the Betulaceae:

In most species the ovules are immature at the time of pollination and the pollen grains germinate and penetrate the styles where they lie dormant for several weeks, until the ovules are ready for fertilization.

The writer has examined several thousand pistils at various stages of development but has never detected any signs of pollen tubes.

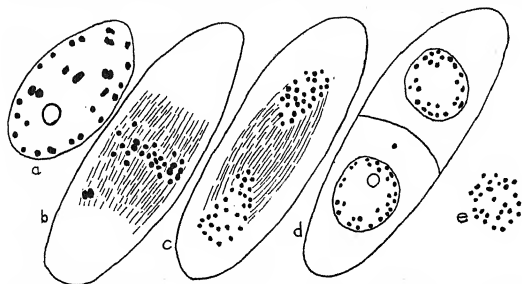


FIG. 1.—a, Diakinesis of embryo sac mother cell; b, metaphase of first division; c, anaphase of first division; d, interkinesis; e, metaphase plate of sporophytic cell showing twenty-eight chromosomes.

Since there is practically no perfect pollen formed, the absence of pollen tubes is to be expected unless pollen from the closely related *Alnus incana* could function. In the ovaries of *A. incana* pollen tube development is readily detected.

Embryo sac formation is initiated about mid-July. Text fig. 1a shows the nucleus of a typical embryo sac mother cell at diakinesis. Chromosome pairs characteristic of this stage are very scarce. This condition is identical with that in microsporogenesis (6), and indicates a heterozygous origin. The metaphase of the first division is shown in text fig. 1b. Most of the chromosomes are single. Apparently they split in halves at this division and reduction fails completely. Text fig. 1c shows the anaphase of the first division with

nearly the full chromosome complement at each pole; *d* is the interkinesis and again shows that reduction does not take place; *e* is the metaphase plate of a sporophytic ovulatory cell showing the chromosome number to be twenty-eight.

The archesporium is of the massive type, not being limited to the production of but one embryo sac mother cell (text fig. 2). A very common condition in young ovules is shown in *b*; one embryo sac is mature and another is forming just above it. Occasionally three and four embryo sacs are formed in the same ovule. Text fig. 2*a* shows

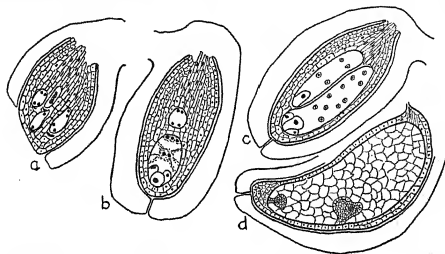


FIG. 2.—*a*, Ovule with four embryo sacs; *b*, *c*, ovule with two embryo sacs; *d*, ovule with embryo from diploid egg and embryo from nucellar budding.

one embryo sac mother cell in the spireme state and three embryo sacs in later stages of development. Free nuclear division of the endosperm initials in two embryo sacs previous to the division of the egg cell is seen in *c*. The several embryo sacs may all produce viable embryos, as will be seen later.

Although no pollen tubes have been seen in thousands of pistils, such evidence is of course inconclusive. Twenty-four clusters of the pistillate aments were bagged about a week before pollination time. According to the suggestion of Professor EAST, old photographic film was freed of the emulsion, rolled into tubes, and the edges stuck with 6 per cent celloidin. These transparent tubes were then slipped over the clusters of fertile catkins and the ends plugged with cotton. About 6 weeks later the tubes were removed to allow the catkins to enlarge normally without being cramped. Microscopic examination

and germination experiments have proved the seeds from these bagged catkins to be different in no particular from the normally formed seed.

#### PARTHENOGENESIS, APOGAMY, POLYEMBRYONY

There are two or rarely three ovules in the young ovary of *Alnus* seeds. Ordinarily one ovule degenerates, the other forming the embryo which completely fills the seed (fig. 1). An embryo may form and mature in each ovule (fig. 3). The normal embryo has its hypocotyl pointing toward the micropyle and stigmas, since it has arisen from the diploid egg at the micropylar end of the ovule (fig. 1). This typifies more than half of the seeds. Occasionally a seed is seen to have its embryo pointing in the opposite direction, toward the chalaza (fig. 2). This suggests the formation of the embryo from the antipodal cells of the embryo sac (apogamy). As yet the earlier stages of such embryo formation have not been found. It may be a case of nucellar budding such as is referred to later.

Polyembryony is of frequent occurrence. One of the embryos is usually parthenogenetic, being developed from the diploid egg, as the smaller embryo in text fig. 2*d*. All of the stages in parthenogenetic embryo building are demonstrable. At times an apogamic embryo forms from one of the synergids beside the parthenogenetic embryo (fig. 5). The writer's preparations include one seed with three well developed embryos which appear to have arisen from the egg and the two synergids.

Nucellar budding (vegetative reproduction) accounts for the formation of many of the extra embryos. Fig. 4 shows the condition as illustrated in text fig. 2*d* at a later stage of development. Fig. 15 shows two embryos developing from opposite sides of the embryo sac wall. A very young embryo is seen forming in the nucellus wall in fig. 19.

Although reported cases of embryo formation from endosperm material are regarded with doubt (4), it seems that such happens in *Alnus rugosa*. In fig. 6 a small dicotyledonous embryo appears at the micropylar end of the sac, while another embryo is apparently developing in the endosperm of the same sac. There is no connection with nucellar material on either side or above or below, as shown

by serial sections. There is a short structure on the upper end resembling a suspensor, which grades into the endosperm. Since this embryo is clearly surrounded by several layers of endosperm cells on all sides, it must have its origin in that material. Figs. 13 and 17 show embryos which proved to be completely surrounded by endosperm material.

Many of the photographs show seeds with interesting conditions of polyembryony, the origin of which is not always clear. Fig. 7 has an embryo sac with two embryos, the lower one bearing five or seven cotyledonary buds. It may be the result of fusion of several embryos. Fig. 8 shows three well formed embryos in the same embryo sac. In fig. 9 are three embryos in the embryo sac on the right, while in the other ovule is an embryo with four cotyledonary buds. Fig. 10 shows two embryo sacs in the same ovule, each with an embryo. The lower embryo has its hypocotyl at the chalazal end, and may have arisen either from the antipodals or as a nucellar bud. Three embryo sacs in the same ovule, each bearing an embryo, are seen in fig. 11. A peculiar condition appears in fig. 12. There is a single embryo in the upper embryo sac and four linear embryos in the lower sac. Fig. 13 shows five embryos in the same sac. Serial sections show them all to be completely surrounded by endosperm. There are no other embryos in this seed. Two nucellar embryos have arisen opposite each other (fig. 15), the one on the left sending two linear cotyledons around one side of the other embryo. One embryo appears in each of two embryo sacs in fig. 16. The larger embryo has sent a young cotyledon over into the other sac. There are two embryo sacs in the same ovule in fig. 17. The three embryos in the upper sac are all completely surrounded by endosperm (serial sections). Fig. 18 shows two embryo sacs with two embryos in each. The microscope shows the two embryos in the upper sac to be distinct. Three embryos have formed from the chalazal end of the embryo sac in fig. 19. Possibly each has come from an antipodal cell, or they may be from the nucellus. A nucellar embryo is just forming in the upper part of the ovule. Fig. 20 shows two embryo sacs with one embryo in the upper and two embryos in the lower sac. There are three embryos in the same embryo sac in fig. 21.



## GERMINATION TESTS

Mature seeds examined under the dissecting microscope showed polyembryony. Fifty seeds were sown on moist soil in the laboratory, and about half of them germinated. Of these, three showed two embryos arising from each seed. In one case the rapid elongation of one of the hypocotyls carried the seed into the air. The smaller hypocotyl stopped elongating when it lost contact with the moisture, and this embryo died before the seed dropped back to the ground. The other embryo is developing well. The two other cases of two embryos germinating from one seed are both living and developing normally. There has been but one cotyledon on one of the embryos from the start but it is in a healthy condition.

Mature seeds from the catkins which were bagged, thus preventing any pollination which might take place, were germinated. These are now growing well and differ in no way from those seeds which were formed naturally. Two of these seeds have produced two embryos each, which are developing normally.

## Discussion

Since further investigation on this subject is to follow, it seems unnecessary to discuss at length previous work on parthenogenesis, apogamy, and polyembryony. The reports of COULTER and CHAMBERLAIN (1) and SHARP (4) treat these subjects well and give reference to most of the important original literature.

There are many well known examples of the phenomena here discussed, except embryo formation from endosperm material. Although this appears to have been demonstrated, it is hoped to detect the early stages of such embryo formation.

Polymorphism, irregularities of meiosis in both microsporogenesis and macrosporogenesis, parthenogenesis, apogamy, nucellar budding, and polyembryony all suggest a hybrid origin for *Alnus rugosa*. ERNST (2) applies this theory widely throughout the plant kingdom. Much cytological and genetical research lends strong support to his theory.

## Summary

1. *Alnus rugosa* is polymorphic.
2. Due to irregularities of microsporogenesis there is practically

no perfect pollen formed. No pollen tubes have been seen in any of thousands of ovaries examined.

3. The plant sets an abundance of viable seeds. Bagged seeds form embryos just as natural seeds do.

4. There is no reduction in chromosome number during macrosporogenesis.

5. From one to four embryo sacs may form in one ovule. Embryos arise from the diploid egg (parthenogenesis) and by nucellar budding. They are also suspected of arising from the synergids, the antipodals, and the endosperm, although the initial stages have not yet been observed. From one to five embryos may mature in one embryo sac. Several embryos may mature in each of two embryo sacs in the same ovule.

6. Germination tests of naturally formed and bagged seeds proved equally successful.

7. Two embryos from the same seed can both develop to normal seedlings.

8. Polymorphism, irregular meioses, parthenogenesis, apogamy, nucellar budding, and polyembryony point to a hybrid origin.

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#### LITERATURE CITED

1. COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of angiosperms. Appleton and Co. New York. 1903.
2. ERNST, A., Bastardierung als Ursache der Apogamie im Pflanzenreich. Gustav Fischer. Jena. 1918.
3. JEFFREY, E. C., Technical contributions. BOT. GAZ. 86:456-467. 1928.
4. SHARP, L. W., An introduction to cytology. McGraw-Hill Book Co. New York. 1926.
5. SWINGLE, D. B., A textbook of systematic botany. McGraw Hill-Book Co. New York. 1928.
6. WOODWORTH, R. H., Cytological studies in the Betulaceae. II. *Corylus* and *Alnus*. BOT. GAZ. 88:383-399. 1929.





## EXPLANATION OF PLATE IX

FIG. 1.—Normal embryo; hypocotyl at micropylar end of embryo sac.

FIG. 2.—Embryo with hypocotyl at chalazal end, possibly from antipodals.

FIG. 3.—Embryo in each ovule of ovary.

FIG. 4.—Two embryos in one embryo sac; one from diploid egg, other from nucellus.

FIG. 5.—Two parallel embryos in same embryo sac, probably from egg and one synergid.

FIG. 6.—Two embryos in one embryo sac; one from egg, other from endosperm.

FIG. 7.—Peculiar embryo complex; lower embryo with five or seven cotyledonary buds.

FIG. 8.—Three embryos maturing in same embryo sac.

FIG. 9.—Three embryos in same embryo sac at right, one tetracotyledonary embryo at left.

FIG. 10.—Embryo in each of two embryo sacs; larger may be from antipodals.

FIG. 11.—Three embryo sacs with embryo in each.

FIG. 12.—Two embryo sacs, upper with one embryo, lower with four embryos.

FIG. 13.—Five embryos in same embryo sac all apparently from endosperm.

FIG. 14.—Two embryos in same embryo sac; one from egg, other from nucellus.

FIG. 15.—Two opposed embryos from nucellar wall.

FIG. 16.—Two embryo sacs with embryo in each; cotyledon of one embryo extending into other.

FIG. 17.—Two embryo sacs, upper with three small embryos from endosperm.

FIG. 18.—Two embryo sacs with two embryos in each (upper two not connected).

FIG. 19.—One embryo sac with three embryos from antipodal region and one young embryo just forming in nucellus.

FIG. 20.—Two embryo sacs with one embryo in upper and two in lower sac.

FIG. 21.—One embryo sac with three embryos maturing.

## BORON AS AN ESSENTIAL ELEMENT FOR HEALTHY GROWTH OF CITRUS<sup>1</sup>

A. R. C. HAAS

Although small amounts of boron are injurious to citrus species,<sup>2</sup> the presence of still smaller amounts may be harmless and even essential for their healthy growth. While boron has been shown by many investigators to be an essential constituent of a rather large and increasing number of plants, it has not previously been shown to be so for citrus.

For the past several years the writer has been employing boron as one of the elements of which traces have been added to the culture solution for the successful growth of citrus plants in water and sand cultures. The culture solution was made up from the following salts: sodium chloride, magnesium sulphate, potassium nitrate, calcium nitrate, potassium acid phosphate, and ferric tartrate. The composition of the culture solution expressed as parts per million was as follows:

| NA | K   | CA  | Mg | FE | NO <sub>3</sub> | CL | SO <sub>4</sub> | PO <sub>4</sub> | TOTAL |
|----|-----|-----|----|----|-----------------|----|-----------------|-----------------|-------|
| 7  | 185 | 159 | 54 | 1  | 718             | 10 | 216             | 105             | 1455  |

The concentration of boron used in the culture solution was 0.2 parts per million. In addition to the boron, a similar concentration of aluminum, iodine, titanium, bromine, strontium, lithium, manganese, and ammonium was used. When traces of all of these elements were present in the culture solution an excellent growth resulted, whereas when they were omitted the growth was poor. In general the symptoms of decline may be described as follows. The leaves curled downward along the midrib, their color being a brownish or yellowish green, often with a yellowing along the midrib; the midrib or veins in many cases were conspicuous, corky, and split; and there was a progressive loss of affected leaves in a basipetal di-

<sup>1</sup> Paper no. 207, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station.

<sup>2</sup> KELLEY, W. P., and BROWN, S. M., Boron in the soils and irrigation waters of Southern California and its relation to citrus and walnut culture. *Hilgardia* 3:445-458. 1928.

HAAS, A. R. C., Toxic effect of boron on fruit trees. *BOT. GAZ.* 89:200-204. 1930.  
*Botanical Gazette*, vol. 89]

rection. In severe cases there was a tendency toward "multiple bud" formation, due to new twigs dying when barely visible. The bark of the internodes of the basal part of the branch may split, when an amber-colored gum oozes out, the crack eventually widening so that the woody tissue is exposed. In these severe cases the apical portion of the branch dies back. The gumming differs from that characteristic of "exanthema" or "die-back," in which the gum pockets are at the leaf nodes. There is a marked reduction or even absence of flower production. The roots become dark brown, fail to elongate, and in advanced cases the rootlets decay. The addition of traces of these elements to the culture solution changes the state of the trees from one of decline to one of vigorous growth. In a few weeks it is impossible to find evidence of the former deficiency symptoms.

It is relatively much simpler to show that a small amount of boron is injurious to citrus than to show that the presence of a mere trace is essential to the good health of the tree. The problem, therefore, was to determine which elements added in traces to the culture solution were responsible for the improved growth.

The water used for the culture solution was distilled from a tin-lined copper still and stored in a copper tank lined with an electrolytic covering of tin. It is possible, therefore, that the water may have contained extremely small amounts of copper and tin. The sand cultures were grown in asphalted, galvanized iron containers, so that the roots had access to zinc. Copper, tin, and zinc were therefore not considered among the traces of elements added to the culture solution, although their possible importance is not being overlooked in further studies upon citrus nutrition.

In every case one grapefruit, one lemon, and one Valencia or Navel orange tree was included in each series of cultures. The galvanized iron containers were approximately 20 inches in diameter, 24 inches deep, and contained approximately 400 pounds of pure silica sand.

The first series received the culture solution without the traces of elements added. The tops of these trees in the course of about two years showed typical symptoms of decline. In the second series, where the culture solution contained traces of all nine elements,

growth was excellent. In other series of cultures all but one of the elements were added to the culture solution, a different one being omitted in each series. In each of these series the growth was excellent except (in cultures) where boron was omitted. The plants in these cultures to which no boron was added showed symptoms identical with those exhibited by plants in cultures receiving the culture solution alone. It is evident, therefore, that citrus plants do not grow satisfactorily without boron, and that traces of the other eight elements added to the culture solution have no visible effect.

Recently boron was added to a series of cultures which for the past two years have received none of the nine elements. Although the plants showed the symptoms ascribed to boron deficiency, within a week after traces of boron were added growth became active. It is known that citrus trees, which are in a declining state as a result of malnutrition, respond very slowly, if at all, to corrective treatments. This is due to the fact that such trees have lost most of their leaves, and the few remaining abnormal ones are unable to furnish the root system with sufficient food for proper growth.

It is of interest to consider sand cultures of citrus trees in 12-gallon earthenware containers which received the culture solution without the addition of the traces of elements previously mentioned. Under these conditions growth was excellent, indicating that the trees may obtain an adequate supply of boron from the vessel for the maintenance of good growth. Water cultures of leafy lemon twig cuttings have been grown successfully in Swedish enameled shallow mixing bowls without the addition of any of the elements in addition to the culture solution. On the other hand, in sand cultures in galvanized iron containers such a solution gave poor results. A booklet published by the American Potash and Chemical Corporation at Trona, California, 1928, states:

Potters, vitreous enamelers, and agateware makers use borax extensively in fluxing the enamel and in glazing the pottery; 27 per cent by volume of the mixture forming the white coating on bathtubs and plumbing fixtures is borax.

It appears, therefore, that the enameled mixing bowls used and certain types of culture vessels may supply boron in amounts adequate for the healthy growth of citrus.



In water cultures the root decline usually precedes foliage decline. In sand cultures the symptoms of boron deficiency are quite obvious at an early stage from the appearance of the foliage, so that the trouble may be diagnosed in time to save the plants, as was the writer's experience with several hundred trees. Foliage symptoms have been observed in citrus orchards that were very similar to those artificially produced in boron-deficient sand cultures. It is quite possible, therefore, that a boron deficiency exists in some orchards. This possibility is being investigated.

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## BRIEFER ARTICLES

### CELLOIDIN TRANSFER METHOD FOR THIN ROCK SECTIONS

(WITH ONE FIGURE)

It is usually desirable to transfer sections of rock ground thin for microscopical observation from the glass slides on which they are ground and polished to other slides free from abrasions or other undesirable effects of grinding. In attempting a transfer the thin rock section is frequently broken. The following simple method, by means of which transfers may be made with a minimum of loss, is the utilization of a cellulose film transfer as worked out by LANG and ASHBY<sup>1</sup> in another connection.

1. Prepare in the usual way the rock specimen to be ground. In petrifactions, sections of rock 1-2 mm. thick may be cut by means of a rapidly rotating thin metal disk, the edge of which has been treated with diamond dust.

2. Grind and polish one surface of the specimen, and flood with a thin solution of celloidin in ether-alcohol. A solution made by dissolving 1 gm. of a standard commercial celloidin in a solution of 50 cc. of ether and 50 cc. of 100 per cent alcohol is satisfactory. Probably a solution of cellulose acetate in amyl acetate as used by WALTON<sup>2</sup> would serve as well.

3. Allow the solution to dry thoroughly on the surface of the rock. The evaporation of the ether-alcohol will leave a thin film of celloidin closely adhering to the polished surface of the specimen. If this film wrinkles and loosens, the solution used is not sufficiently dilute and additional ether-alcohol should be added.

4. Mount the specimen in Canada balsam on a glass slide preparatory to grinding thin with abrasive powder. Natural Canada balsam which has been heated over a water bath until it is perfectly hard when cooled has been found to be more satisfactory than Canada balsam which has been dissolved in xylol. In mounting the specimen in balsam, the surface covered with the celloidin film is placed next to the slide (fig. 1).

<sup>1</sup> LANG, W. H., A cellulose film transfer method in the study of fossil plants. *Ann. Botany* 40: 710. 1926.

<sup>2</sup> WALTON, J., Recent developments in palaeobotanical technique. *Congrès Stratigraphie Carbonifère. Heerlen. 1927 (1928).*

5. Grind the exposed surface of the rock with abrasive powder until the section is sufficiently thin, wash and polish to remove all traces of the abrasive, and dry the section thoroughly.

6. Place the glass slide with the thin section in a shallow pan and cover with xylol. Place the pan on a warming table and warm until the balsam in which the rock has been mounted is dissolved sufficiently to loosen the section from the glass slide. Final traces of abrasive powder may be removed. Allow sufficient time for this step. Mechanical assistance in loosening the section will cause frequent disasters. Normally the presence of the celloidin film will prevent the section from breaking and will allow the following necessary steps.

7. After the section has been loosened from the slide, drain off all excess xylol, and while still on the warming table cover with a generous amount of a very dilute solution of Canada balsam in xylol. The balsam will flow



FIG. 1.—Diagram showing rock section (a) with celloidin film (b), mounted in Canada balsam (c) on glass slide (d), ready to be ground thin and polished.

quickly under the rock section, separating it from the glass slide and allowing the section to be moved with ease. Now place over the section a cover slip of the kind desired in the permanent mount. This will act as a guide in the actual transfer to the second slide.

8. The transfer is made to a warm glass slide on which has been placed a small amount of Canada balsam. Hold the slide carrying the rock section and cover slip in a vertical position above the second glass plate and allow the section, guided by the cover slip, to slide from the one to the other. Arrange the section and cover slip as desired and place in drying oven until the balsam is sufficiently hard to insure safe handling.

If the rock material is especially friable, an added precaution against its breaking during the transfer may be taken. After the rock section has been ground sufficiently thin and the abrasive powder removed, cover the exposed surface with the celloidin solution, allow it to dry, and then proceed as indicated. The celloidin clears perfectly in xylol, and its presence in the permanent mount, even covering both surfaces of the thin section, does not in the least hinder microscopical observation of sections of petrifactions.—J. H. HOSKINS, *University of Cincinnati, Cincinnati, Ohio.*

## CHROMOSOME NUMBERS IN THE CUCURBITACEAE

The chromosome counts of species of Cucurbitaceae recorded in the literature at the present time represent principally the species and varieties of economic importance. During the last two years I have been interested in making a collection of the wild and cultivated species of the family occurring in the temperate zone, and a study of the chromosome

TABLE I

| SPP.   | N     | 2N    | REPORTED BY                        |
|--|-------|-------|------------------------------------|
| <i>Bryonia alba</i> .....                      | 10    | ..... | von Boenicke, <sup>1</sup> Meurman |
| <i>B. dioica</i> .....                         | 10    | ..... | Strasburger, Meurman               |
| <i>Benincasa hispida</i> .....                 | ..... | 24    | Author                             |
| <i>Bryonopsis laciniosa</i> .....              | ..... | 24    | Author                             |
| <i>Citrullus vulgaris</i> .....                | 11    | ..... | Kozhukhow, Author                  |
| <i>Coccinia hirtella</i> .....                 | ..... | 24    | Author                             |
| <i>Cucumis sativus</i> .....                   | 7     | ..... | Kozhukhow                          |
| <i>C. melo</i> .....                           | 12    | ..... | Kozhukhow, Author                  |
| <i>C. dipsaceus</i> .....                      | ..... | 24    | Author                             |
| <i>C. metuliferus</i> .....                    | ..... | 24    | Author                             |
| <i>C. myriocarpus</i> .....                    | ..... | 24    | Author                             |
| <i>Cucurbita ficifolia</i> .....               | ..... | 42    | Author                             |
| <i>C. foetidissima</i> .....                   | ..... | 42    | Author                             |
| <i>C. palmata</i> .....                        | ..... | 42    | Author                             |
| <i>C. pepo</i> .....                           | 12    | ..... | Lundegårdh                         |
| <i>C. pepo</i> var. <i>pomiformis</i> .....    | 20    | ..... | Kozhukhow                          |
| <i>C. pepo</i> var. <i>citrullina</i> .....    | 21    | ..... | Kozhukhow                          |
| <i>C. moschata</i> .....                       | 24    | ..... | Kozhukhow                          |
| <i>C. maxima</i> .....                         | 24    | ..... | Kozhukhow                          |
| <i>C. maxima</i> .....                         | 20    | ..... | Castetter                          |
| <i>Cyclanthera pedata</i> .....                | ..... | 32    | Author                             |
| <i>Ecballium elaterium</i> .....               | ..... | 24    | Author                             |
| <i>Echinocystis (micrampelis) lobata</i> ..... | 16    | ..... | Kirkwood                           |
| <i>Lagenaria vulgaris</i> .....                | ..... | 24    | Author                             |
| <i>Luffa acutangula</i> .....                  | ..... | 26    | Author                             |
| <i>L. marylandica</i> .....                    | ..... | 26    | Author                             |
| <i>Melothria punctata</i> .....                | ..... | 24    | Author                             |
| <i>Mormordica charantia</i> .....              | ..... | 22    | Author                             |
| <i>Sicyos angulata</i> .....                   | ..... | 24    | Author                             |

<sup>1</sup> See literature cited at end of paper for references.

number and morphology of these forms is being carried on with a view to contributing more fully to our knowledge of this family. The list of species being studied is by no means complete, but representatives from practically all genera of the temperate zone are included. It is hoped to secure knowledge of the range of chromosome number in the family as a working basis for further determinations of particular genera and species.

The work being conducted at the University of California on the Cucurbitaceae is concerned chiefly with two problems: (1) a survey of

chromosome numbers in species not heretofore studied; and (2) a cytological study of the varieties of cultivated Cucurbitaceae as a basis for the interpretation of results of breeding experiments now being carried on.

Table I gives a complete summary of the reported chromosome numbers in the Cucurbitaceae.

It will be noticed that there is a lack of agreement in the numbers reported by various investigators for the species and varieties of the genus *Cucurbita*. The purpose of the present work is also to take up a thorough treatment of the somatic chromosomes of the species and varieties of this important genus, in an effort to determine whether or not there is a range of variation of chromosome numbers as suggested by these earlier results. The somatic chromosomes are being studied in root cells, and in most cases the haploid number will be determined from pollen mother cells as a check against the somatic count.—J. W. MCKAY, *University of California, Berkeley, Calif.*

#### LITERATURE CITED

1. BOENICKE, L. VON, Zur Kenntnis der Prophasen der heterotypischen Teilung einiger Pollenmutterzellen. Ber. Deutsch. Bot. Ges. 29:59-65. 1911.
2. CASTETTER, E. F., Microsporogenesis in *Cucurbita maxima*. Amer. Jour. Bot. 13:1-10. 1926.
3. KIRKWOOD, J. E., Some features of pollen formation in the Cucurbitaceae. Bull. Torr. Bot. Club 34:221-242. 1907.
4. KOZHUKHOW, S. A., Karyotypische Eigentümlichkeiten der kultivierten Cucurbitaceae. Bull. Appl. Bot. Pl. Breed. 14:89-96. 1925.
5. LUNDEGÅRDH, H., Zur Kenntnis der heterotypischen Kernteilung. Arch. Zell. 13:145-157. 1914.
6. MEURMAN, O., The chromosome behavior of some dioecious plants and their relatives with special reference to sex chromosomes. Soc. Sci. Fennic. Commentat. Biol. 2:1-104. 1925.
7. STRASBURGER, E., Über geschlechtbestimmende Ursachen. Jahrb. Wiss. Bot. 48:427-520. 1910.

# CURRENT LITERATURE

## BOOK REVIEWS

### Outlines of biochemistry

There has been great need of a good text on biochemistry for the student of the non-medical biological sciences. An attempt to meet this need has been made by GORTNER,<sup>1</sup> who has brought into book form the experiences of years of research and teaching in the division of agricultural biochemistry of the University of Minnesota.

The seven sections of the text present (a) the colloid state of matter; (b) proteins; (c) carbohydrates and allied compounds; (d) tannins; (e) plant pigments; (f) fats, lipides and essential oils; (g) biocatalysts. The first of these sections consists of ten chapters on colloidal phenomena. This is one of GORTNER's favorite fields, and it is handled in characteristic fashion. It is an indication of the importance which the author attaches to these colloidal relations, that more than a third of the space of the entire volume is devoted to them. It is on the whole an attractive and stimulating summary of the physics and chemistry of matter in colloidal subdivision.

The section on proteins consists of ten chapters also, and occupies about one-fourth of the volume. The scope of treatment is indicated by the following abbreviated chapter headings: amino acids; polypeptides; analysis; structure and isomerism; classification; properties of protein systems; reactions with acids and bases; metabolism; biological reactions; and nitrogen bases.

The carbohydrates and their congeners are treated less extensively, but with sufficient detail as to the chemistry of the group. The first chapter in this section discusses the problems of optical rotation, isomerism, the importance of the carbohydrates, synthesis of carbohydrates in nature, the first sugar formed in the process of photosynthesis, the energy relations of photosynthesis, and the classification of the carbohydrates.

To the reviewer the book seems better fitted to the needs of students of zoology, botany, agriculture and its related biological fields than any text on biochemistry hitherto produced. Much of the text has previously been given in the form of class lectures, and the author and publishers deserve the thanks of students of biology for presenting the material in permanent form.—C. A. SHULL.

### Hydrogen-ion concentration in plant cells and tissues

The second volume of this series<sup>2</sup> is a monograph on hydrogen-ion concentration with special reference to plant materials, by SMALL, who has been a student

<sup>1</sup> GORTNER, R. A., *Outlines of biochemistry*. 8vo. pp. xvi+793. New York: John Wiley and Sons. 1929.

<sup>2</sup> SMALL, J., *Hydrogen-ion concentration in plant cells and tissues*. *Protoplasma Monographien* Vol. II. 8vo. pp. xii+421. Berlin: Gebrüder Borntraeger. 1929.

in this field for a number of years. The work is divided into three sections, the introduction, methods, and results. The introduction considers the hydrogen-ion concentration problems in connection with stability of the proteins, their characteristic reactions, and the effects of variations of pH upon the living and reserve proteins; enzyme action; the buffer systems in cells; variations in pH of sap, wall, and protoplasm, etc.

Part II on methods contains eight chapters, with discussions of the hydrogen electrode measurements, quinhydrone, and micro-hydrogen electrodes; comparator indicator methods, capillator indicator, special indicator methods, range indicator methods, and buffer determinations.

The results are recorded first in the form of a general survey of tissue reactions, and diurnal and seasonal changes in reaction; then such special cases as follows: the sunflower, broad bean, potato, succulents, etc. The final chapters are devoted to the relations of protoplast, cell sap, and cell walls to pH, buffers and buffer indexes in plants, and a restatement of the problems at the close. There are several appendixes, on Embden's phosphate method, organic acid analysis, and supplementary references. The literature citations occupy 25 pages, and provide an excellent guide to the past literature of the subject.

The book is full of information, and will be found useful especially by students who have to supplement inadequate chemical background by recourse to books upon the subject of hydrogen-ion measurements. The reviewer notes the criticisms of some American work in various places through the volume. After all, there is nothing sacred about the way in which we present our ideas. A certain amount of standardization in presentation is a good thing, but it does not need to be made a fetish.

Anyone who wishes to carry on investigations in this field should have mastered the material presented in such a book before he begins.—C. A. SHULL.

#### General botany

In the second edition of HOLMAN and ROBBINS' well known and widely used textbook of general botany,<sup>1</sup> published in the fall of 1927, the organization and method of treatment of the subject are essentially the same as in the first edition, which was published in 1924, and reviewed in the March, 1925, issue of this journal.

The principal changes in the second edition are: the introduction into the first and second chapters of brief statements of certain physical and chemical principles with which the student should be acquainted in order to understand the fundamentals of plant physiology; placing of the chapter on the plant body before the chapter on the cell instead of after it, as it was in the first edition; the introduction at the end of Part I of a chapter on the relation of the plant to its environment in place of the "summary" chapter of the first edition; the

<sup>1</sup> HOLMAN, R. M., and ROBBINS, W. W., A textbook of general botany. 8vo. pp. xiii+624. figs. 415. New York: John Wiley & Sons. 1927. \$4.

introduction in the chapter on the spermatophyta of an account of the principal tendencies in the evolution of the angiosperms, based upon CLEMENT's presentation of the Besseyan system; various changes throughout the text prompted by recent advances in botanical knowledge, particularly in histology and physiology; the addition of more than fifty new illustrations and the redrawing, entirely or in part, of about the same number of figures used in the first edition.

The second edition, like the first, is written in clear style, including much detail, and is amply illustrated.—J. M. BEAL.

#### Canary Islands plants

The Canary Islands have long had a special fascination for botanists because of their position, abundant endemics, and the close affinity of their flora to that of Tertiary times. In spite of this attraction their flora has been rather imperfectly known. The present monograph,<sup>1</sup> however, will do much to advance our knowledge of this remarkable vegetation.

The age and climate of the islands are examined and the influence of climate on the vegetation considered. There follows a description of the vegetation of seven individual islands. Then comes the major portion of the volume in the special investigations of the author on the various species. Each species is carefully considered and its peculiarities described. These descriptions, together with remarkably fine plates from photographs by the author, make a detailed knowledge possible. It is a valuable study presented in most attractive form.—G. D. FULLER.

#### Tropisms

This volume<sup>2</sup> is one of a series of monographs reviewing and bringing to date various branches of science. The first part of the book summarizes what is known of tropisms in both plants and animals, and there is an extensive bibliography at the end of each of the six chapters. The bibliography of phototropism of plants occupies ten pages and contains some 150 citations. The second part of the volume is concerned with a critical examination of the theories of JENNINGS and of LOEB; while the third part contains a general discussion of transmission of stimuli and its relation to philosophy and psychology.

Such a useful work of reference deserves to be better printed and more attractively bound.—G. D. FULLER.

#### The one-year course in general biology

An impressive text for a first-year college course in general biology has been prepared by PLUNKETT.<sup>3</sup> In temporary form, this book has already received a

<sup>1</sup> BURCHARD, OSCAR, Beiträge zur Ökologie und Biologie der Kanarenpflanzen. Bibliotheca Botanica. Edited by L. DIELS. 4vo. Heft 98: pp. 262. pls. 86, map. Stuttgart. 1929.

<sup>2</sup> ROSE, MAURICE, La question des tropismes. Les problèmes biologiques. XIII. pp. vii+469. figs. 90. Paris: Les Presses Universitaires de France. 1929. 15 fr.

<sup>3</sup> PLUNKETT, C. R., Outlines of modern biology. 8 vo. pp. vii+711. figs. 198. New York: Henry Holt & Co. 1930. \$3.75.



five-year trial with classes of over a thousand students each at New York University. The basis of organization is the concept of the living organism as a physico-chemical mechanism. Accordingly the twenty-seven chapters are grouped into five sections: I. protoplasm; II. nutrition; III. response; IV. reproduction; V. evolution. Illustrations are chosen freely from plants, lower animals, and man, phylogenetic considerations being reserved for the last few chapters. An index and a short list of selected references appear at the end.

Admittedly the contents are rather full for an eight-months' college course, and perhaps a little more difficult than is customary in biology. Deliberately the student is exposed to more than he is expected to reproduce in examination. Yet there are elements of flexibility which should enable each teacher to use as much or as little of the contents as are appropriate for his own group. Although the text does not commit the teacher to any one style of laboratory program, an accompanying laboratory manual by H. J. FRY is promised shortly.

In this day of survey courses this book should undoubtedly find a place. It is more highly synthetic and more dominantly physiological than any other first-year biology the reviewer has seen.

Almost simultaneously there appears a revised edition of a similar volume by SCOTT.<sup>1</sup> This represents the more orthodox handling of first-year college biology. The first half of the book contains a detailed and systematic treatment of plant and animal phylogeny, while the last half is organized into: the cell; embryology; histology; comparative anatomy; reproduction; physiology; genetics; distribution of organisms in time and space; evolution; the biology of man. A comprehensive glossary and index are included, while a generous list of selected references appears at the end of each chapter.—M. C. COULTER.

#### Phylogeny of plants

A volume<sup>2</sup> with a pretentious title has just appeared, and any presentation of it by one man is sure to be stronger in the field of that man's research. Outside of that field he must rely largely upon literature. The conclusions in any treatment of phylogeny are based upon paleobotany, experiment, and comparative morphology. The law of irreversibility, which has been established principally by vertebrate paleontologists, is well stated.

The first part of the book deals with the morphology of plants, from the Cyanophyceae to the orchids. Some of it is well presented, especially the morphology of fossil plants; but in the life history diagrams there are some surprising inaccuracies. The tetraspores of *Dictyota* are motile and the sperm has two cilia. The egg of *Laminaria* is figured with cilia. Investigations here are none too complete, but the behavior shows that this egg is of the non-motile type. The sperm is figured with two cilia, which is probably correct; but, so far

<sup>1</sup> SCOTT, G. G., The science of biology: an introductory study. 2d ed. 8vo. pp. xx+633. figs. 390. New York: Thomas Y. Crowell Co. 1930. \$3.75.

<sup>2</sup> ZIMMERMAN, W., Die Phylogenie der Pflanzen, ein Überblick über Tatsachen und Probleme. 8vo. pp. xi+452. figs. 250. Jena: Gustav Fischer. 1930.

as the reviewer knows, cilia have not yet been discovered. The egg of *Fucus* is shown with two cilia. The author's knowledge of life histories should be more accurate, if he is to draw important conclusions from them.

In looking through the numerous illustrations, it is evident that many are incorrectly acknowledged. Some, which the reviewer did not make, are credited to COULTER and me; and a number of my illustrations of gymnosperms are credited to another author; some of RENAULT's figures are credited to ARBER, etc. The author evidently merely consulted general accounts, in which the illustrations may have been properly acknowledged, and carelessly credited that source instead of the original account. Unfortunately the present book is not the only offender of this sort.

Some conclusions, as expressed in diagrams, may be mentioned: the Flagellates have given rise to the fungi; then in succession to the red, brown, and green algae, with the possibility that some of the fungi may have come from the red and green algae. The Cyanophyceae, which are referred to as having no nuclei ("Kernlose Thallophyten"), are a parallel development and have given rise to the bacteria. The Pteridospermae, Cordaitales, and Coniferae are derived from the Filices; but there is a suggestion that the Coniferae may have come from the Lycopsidea. Both Ginkgoales and Cycadales are derived from the Pteridospermae; and the Cycadales have given rise, in the Triassic, to both the Bennettitales and angiosperms. The Gnetales are derived from the Bennettitales.

Until much more is known about development, and especially the amount of reliance which can be placed upon similarity of structures in determining relationships, any phylogenies will be tentative. The subject is advancing and the present book makes some contribution, especially where it touches upon paleobotany, ancient geographic distribution, and the principles of phylogeny.—  
C. J. CHAMBERLAIN.

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